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FURTHER INVESTIGATIONS ON EXPERIMENTAL ATHEROSCLEROSIS

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Because blood vessel lesions resembling human atherosclerosis have never been produced experimentally by any means except cholesterol feeding, a good deal of emphasis has been placed on cholesterol as the important etiologic factor in human atherosclerosis also.[1] There are, however, important differences in pathology and pathogenesis between experimental and human atherosclerosis.[2] This, combined with the fact that no one has ever succeeded in producing the experimental disease by cholesterol feeding in any animals but the rabbit and guinea-pig, has led to the growing conviction that there must be some other contributing factor. One possible mechanism is suggested by Duff, who describes a primary injury to the arterial walls in cholesterol-fed rabbits which favors precipitation of lipids in the walls. The nature of the factor causing the injury is, however, still entirely speculative.

For several years considerable work has been done in this laboratory in the hope of demonstrating some such factor. In attempting to explain the primary changes both in human and experimentally produced atherosclerosis, two possibilities were borne in mind: (1) that there was a change in the permeability of the wall of the vessel in the neighborhood of the internal elastica which prevented the normal passage of large molecules such as proteins and lipids from the intima into the media without interfering with the passage of water and crystalloids, and (2) that there was an increased permeability of the intima without other injury to the vessel wall. In both instances there may occur a deposition of relatively large amounts of lipids and of coagulated and

This investigation has been aided by a grant from the Josiah Macy Jr. Foundation.

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1. Anitschkow, N., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, chap. 10.

2. Duff, G. L.: Arch. Path. **20**:81 and 259, 1935.

finally hyalinized protein. It was with these two possibilities in mind that the experiments to be described were planned. Though we shared the growing prejudice against rabbits for this type of work, we felt that feeding cholesterol to rabbits represented the most obvious starting point available at present. The general plan, therefore, was to feed young adult rabbits [3] cholesterol for short periods of time—seldom longer than thirty-one days—and combine other injurious procedures in various ways with the feeding. Since by the method employed [4] feeding cholesterol alone (though it produced a high level of cholesterol in the blood) practically never produced lesions in this short time, it seemed that any effect of the additional procedures should be fairly clearcut. With hypercholesterolemia the conditions appeared favorable for the production of fatty deposits in the aorta if there was damage to the intima resulting in increased permeability.

RABBIT EXPERIMENT 1

The aorta of the rabbit has long been known to be particularly susceptible to a variety of noxious agents. Of these bacteria and their toxins were among the first to be used in the supposed production of arteriosclerosis.[5] The lesions so produced are now generally regarded as either medial or inflammatory unless—as happened in Saltykow's early experiments—the diet also contained cholesterol. There is evidence, however, both in Saltykow's work with staphylococci [6] and in the later experiments of Benson and co-workers with streptococci [7] that bacterial injections may accelerate and intensify the formation of the cholesterol lesions. But there is no recent report of the possible effect of bacterial toxins on the development of these lesions. In experiment 1 of this series a streptococcus toxin was employed for this purpose.

Procedures.—Intravenous injection of streptococcus toxin [8] was combined with cholesterol feeding in six rabbits:

(*a*) Two rabbits were each fed 1 Gm. of cholesterol daily for three weeks in the manner described, and were then given nine increasing intravenous doses of toxin on consecutive days (beginning with 0.1 cc. and increasing to 10 cc.).

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3. The rabbits used were chiefly from the stock of gray chinchillas raised on the farm kept by the Animal Care Department of the medical school.

4. One gram of dry cholesterol was mixed with the basic diet three, four or six times per week. The latter consisted of a mixture of whole and rolled oats, with occasional greens.

5. Boinet and Romary: Compt. rend. Soc. de biol. **58**:1153, 1906. Boinet: Arch. de méd. expér. et d'anat. path. **9**:902, 1897. Klotz, O.: Brit. M. J. **2**:1767, 1906. Saltykow, S.: Centralbl. f. allg. Path. u. path. Anat. **19**:321 and 368, 1908.

6. Saltykow, S.: Beitr. z. path. Anat. u. z. allg. Path. **57**:415, 1913.

7. Benson, R. L.; Smith, K. G., and Semenov, H.: Arch. Path. **12**:924, 1931.

8. Dr. Alvin Coburn of the Presbyterian Hospital very kindly supplied the streptococcus toxin. It was made from Dochez' N. Y. 5 strain according to the procedure outlined by Wadsworth (Standard Methods, Baltimore, Williams & Wilkins Company, 1927, pp. 88 and 328).

The last three doses produced shock in both rabbits. Cholesterol feeding was continued, and the rabbits were killed after their last dose of toxin.

(*b*) Four rabbits were each given six increasing doses of toxin (from 0.1 to 6 cc.) on consecutive days and were then started on cholesterol feeding (as described), injections of toxin being continued, at the upper level, for seven days more in two of the rabbits and for sixteen days more in the other two.

(*c*) As controls two rabbits were fed cholesterol alone for eight and nineteen days, respectively.

Result: No fatty lesions were produced in the aortas of the rabbits in any of these groups. Though the time over which the injections of toxin were given was not very long, it corresponded roughly with that of the average acute streptococcus infection. In infants fatty plaques are sometimes found in the intima of the aorta after even a shorter period of severe infection.[9]

RABBIT EXPERIMENT 2

The question of the effects of abnormal diets on the blood vessels has been argued extensively since Ignatowski[10] first observed atherosclerotic changes in rabbits fed protein foods like meat, eggs and milk. Though the chief injurious factor in these diets has now been definitely established to be their cholesterol content, it is by no means so certain that such abnormal diets do not produce in rabbits other metabolic disturbances which might have a primary effect in injuring the wall of the aorta, thereby favoring precipitation of cholesterol and fats. The possible effect of a continued acid-forming diet[11] on an animal unaccustomed to it was brought out by Nuzum.[12] His rabbits, which acquired extensive arteriosclerosis on a diet of liver, had shown the carbon dioxide in the blood lowered and the urine acid for a period of months. An attempt was made in experiment 2 to produce this effect directly by using an acid-forming salt like ammonium chloride (NH_4Cl).[13]

Procedures.—(*a*) Eight rabbits were given from 30 to 50 cc. of 2 per cent ammonium chloride solution by stomach tube daily and from 3 to 4 Gm. of cholesterol per week for four weeks.

(*b*) Nine rabbits were given ammonium chloride alone in the same daily doses for four weeks.

The serum carbon dioxide was in general lowered 20 to 30 per cent after a week of ammonium chloride feeding, but in most cases tended to return to normal by the end of the month.

Result: No fatty lesions were found in the aortas of these rabbits.

9. Zeek, P.: Arch. Path. **10**:417, 1930.
10. Ignatowski, A.: Arch. de méd. expér. et d'anat. path. **20**:1, 1908.
11. Newburgh, L. H., and Clarkson, S.: Arch. Int. Med. **31**:653, 1923.
12. Nuzum, F. R.; Seegal, B.; Garland, R., and Osborne, M.: Arch. Int. Med. **37**:733, 1926.
13. Koehler, A. E.: J. Biol. Chem. **72**:99, 1927.

RABBIT EXPERIMENT 3

Experiment 3 was planned with a view to reproducing the early fatty lesions so frequently found in children suffering from acute infections with high fever.[14]

Procedures.—(*a*) Three rabbits were subjected to artificial fever and cholesterol feeding. They were placed in an incubator at from 44 to 45 C. (111.2 to 113 F.) for one hour twice daily, during which time their temperatures would rise to from 107 to 110 F. (normal from 101 to 102 F.) to fall rapidly back to normal within one-half hour after they were taken out of the incubator. One rabbit was fed 1 Gm. of cholesterol daily for twenty-nine days and was then given fever for seven days and fever in addition to cholesterol for ten days more.

(*b*) As controls two rabbits were fed cholesterol alone for fifteen and thirty-four days.

Results: No fatty lesions were found in the aortas of these animals.

RABBIT EXPERIMENTS 4, 5 AND 6

The choice of procedures in experiments 4, 5 and 6 was determined chiefly by their possible effect on intimal permeability. If, in the pathogenesis of arteriosclerosis there is, as we have postulated, a differential change in the permeability of the inner and outer layers of the wall of the aorta—that is, if the permeability of the intima is increased while that of the rest of the wall remains normal—then this condition, particularly if combined with an increased lipid content in the tissue lymph passing through the wall from the lumen of the vessel, should favor deposition of the lipid at the natural barrier between the two parts of differing permeability. Morphologically, the demarcation of the early fatty plaque by the internal elastic membrane strongly suggests that some such factor must come into play.

In experiments 4 and 5 peptone injections and anaphylactic shock were used because, like histamine, they increase capillary permeability.[15] Experiment 6 was suggested by the work of Chini,[16] who in dye diffusion experiments showed that when uric acid was injected intraperitoneally along with congo red the subcutaneous and periarticular tissues became much more deeply colored than when dye alone was injected. The same was true for subcutaneous injections, and Chini concluded that uric acid also increases capillary permeability. It was hoped that a similar effect on intimal permeability might be achieved by using these substances and that the increased permeability might be manifested by an accelerated and more extensive production of cholesterol lesions in the experimental animals.

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14. Ophüls, W., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, chap. 9. Zeek.[9]

15. Sollmann, T.: A Manual of Pharmacology, ed. 3, Philadelphia, W. B. Saunders Company, 1930, p. 475.

16. Chini, V.: J. Exper. Med. **53**:1, 1931.

Procedures in Experiment 4.—(*a*) Six rabbits were given daily intravenous injections of peptone solution (0.2 cc. per kilogram of a 10 per cent solution of a standard commercial preparation) and were fed cholesterol (from 3 to 4 Gm. per week) for one month. The procedure was repeated on six more rabbits a year later.

(*b*) Six controls were given intravenous injections of peptone alone for one month.

(*c*) Ten controls were fed cholesterol alone (from 3 to 4 Gm. per week) for one month.

Result: Three of the first six rabbits receiving cholesterol and peptone and one of the four controls receiving cholesterol alone showed a few early fatty plaques in the arch of the aorta. When the experiment was repeated the next year, however, none of the rabbits, either in the experimental group or in the control groups, showed any lesions.

Procedure in Experiment 5.—Anaphylactic shock was combined with cholesterol feeding in three rabbits. These animals were sensitized by two series of four daily intravenous injections of 0.5 cc. of horse serum, with a three day rest period between the two series. Shock was produced twelve and nineteen days after their last sensitizing dose. Cholesterol feeding (4 Gm. per week) was started at the beginning of the second week and was continued throughout the period of production of shock.

Result: No fatty intimal lesions were found in the aortas of these rabbits.

Procedures in Experiment 6.—(*a*) Intravenous injection of uric acid was combined with cholesterol feeding in three rabbits. (The uric acid was prepared according to Koehler.[17] The dosage was 12 mg. per kilogram on the first day, 24 mg on the second and 48 mg. per kilogram from the third day to the end of the experiment.) Each rabbit was fed 1 Gm. of cholesterol daily for one week before the injections were begun and throughout the period of administration of uric acid. One received four doses, and two received eleven daily doses of the uric acid.

(*b*) As controls two rabbits were fed cholesterol alone for twenty-eight days.[18]

Result: No fatty lesions were found in the aortas of these animals.

Of all these procedures the only one that gave any suggestive results was that of combined cholesterol feeding and peptone injection. Three of the first six rabbits showed a few early intimal fatty lesions in the arch of the aorta as compared with one of the four controls fed cholesterol alone for the same time. However, since these results could not be repeated the next year, it seems reasonable to conclude only that the lesions in the first experiment were due to better absorption of the cholesterol in both groups.

17. Koehler, A. E.: J. Biol. Chem. **60**:721, 1924.

18. In the six groups of rabbits sixteen controls were fed cholesterol alone for periods varying from eight to thirty-four days, and all of them may justifiably be considered as controls for any one experimental group.

It may be of interest to note here the incidence of spontaneous arteriosclerosis in these groups of rabbits. Of a total of sixty-six rabbits, twenty-two showed the well known medial degeneration of the aorta, and it was about equally distributed between the control and experimental groups.

CAT EXPERIMENTS

In the course of this work with rabbits similar experiments were performed on cats. The fact that vascular lesions cannot be produced in cats by feeding of cholesterol alone [19] indicates that it is difficult to disturb the ordinarily adequate cholesterol metabolism of these animals. Accordingly it was felt that a relatively long exposure to the added injurious agents would be necessary in order to duplicate results produced in rabbits in a shorter time. Most of the cat experiments, therefore, have been long chronic ones.

Procedures in Group 1.—(*a*) Two cats were given intravenous injections of peptone and fed cholesterol. Each cat was given 0.2 cc. of 10 per cent peptone solution per kilogram intravenously and 10 cc. of a cholesterol emulsion (equal to 1 Gm. of cholesterol) in its food daily for eighteen and one-half or twenty-two weeks, respectively. (The emulsion was made by grinding 10 Gm. of cholesterol with 50 cc. of 5 per cent acacia solution in a mortar, adding 50 cc. of peanut oil and mixing till homogeneous.)

(*b*) As controls two cats were fed the stock laboratory diet (cooked meat and vegetable stew).

Result: At autopsy, no changes were found in the aortas.

Procedures in Group 2.—(*a*) In four cats intravenous injections of histamine were combined with cholesterol feeding. Each cat was given 20 cc. of a cholesterol emulsion (equal to 2 Gm. of cholesterol) by stomach tube daily for nine days; then, while cholesterol feeding was continued, each was given a daily intravenous dose of 0.02 cc. of histamine per kilogram (of a solution made by dissolving 1 mg. of histamine in 5 cc. of water) for five, eight, thirteen or sixteen days more, respectively.

(*b*) As controls four cats were given intravenous injections of histamine alone. Two cats received five and six injections, respectively, and two received thirteen and fourteen injections, respectively, on consecutive days. (The dose was the same as that described.)

(*c*) Two cats were given intravenous injections of histamine and fed cholesterol for a long period. The animals were each given 10 cc. of cholesterol emulsion (equal to 1 Gm. of cholesterol) in their food and intravenous injections of histamine as described in (*a*) daily for twenty-two weeks.

Result: The last two cats had increased blood cholesterol, approximately twice the normal, but showed no lesions in the aorta. The cat

19. (*a*) Cirio, L.: Virchows Arch. f. path. Anat. **269**:739, 1928. (*b*) Yuasa, D.: Beitr. z. path. Anat. u. z. allg. Path. **80**:570, 1928.

which received both histamine and cholesterol for thirteen days showed a few small areas of intimal wrinkling and thickening. In the remaining cats the aorta was normal.

Procedures in Group 3.—(*a*) Six cats were fed cholesterol and ammonium chloride. Each cat was fed from 1 to 2 Gm. of ammonium chloride mixed with its food (kibbled bread and salmon, or chopped meat with added calcium gluconate and sodium chloride [NaCl]) daily for five months, and then the same amount of ammonium chloride plus 1 Gm. of cholesterol dissolved in 3 Gm. of butter daily for from eight weeks to ten months.

(*b*) As controls five cats were fed ammonium chloride alone. From 1 to 2 Gm. of this salt was mixed with the food of each daily for from seven weeks to fifteen months. (It was impossible to produce a continued acidosis in cats with ammonium chloride. If given by stomach tube the salt was invariably vomited at once, and when mixed with the food, as it was finally given, it produced only a transitory and slight lowering of the blood carbon dioxide.)

(*c*) As controls three cats were fed the stock laboratory diet.

Result: Two of the cats fed ammonium chloride and cholesterol showed vaguely outlined small yellowish-gray areas in the arch of the aorta, but similar areas were also found in two of the three control cats.

The viscera of the cats fed cholesterol were generally very fatty, and in the long-continued experiments the blood cholesterol was somewhat elevated, in two animals up to twice the normal amount. No significant vascular lesions, however, were present in any of the cats with the exception of one in each of groups *2a* and *3a*. The aorta of one cat in group *2a* contained several small foci of medial degeneration with fibrosis and calcification. In the thoracic aorta of one cat in group *3a* there was present a small opaque gray spot which was found on microscopic examination to represent a thick, fibrous intimal plaque overlying a zone of necrosis in the outer third of the media. In several cats in both the control and experimental groups small areas of intimal wrinkling and thickening with splitting of elastic lamellae were noted. No fatty plaques were found in any of the animals. These experiments, therefore, seem to be only further confirmation of the work of Cirio [19a] and Yuasa,[19b] who found it impossible by feeding cholesterol to produce lesions in the blood vessels of cats comparable to human arteriosclerosis. Nor does administration of a substance like histamine, known to increase the permeability of the vessels, or an excessively acid diet appear to have a predisposing effect.

The absence of a suggestive lead in any of these experiments was disappointing and leaves the nature of the initial change or injury which might favor deposition of lipoids in the intima of the aorta still unknown.

SUMMARY

An unsuccessful attempt has been made to accelerate and increase the development of cholesterol lesions in the aorta of the rabbit by the following injurious procedures added to cholesterol feeding: (1) intravenous injections of streptococcus toxin, (2) ammonium chloride feeding, (3) production of artificial fever, (4) intravenous injections of peptone, (5) induction of anaphylactic shock and (6) intravenous injections of uric acid.

Similarly, no effect on the blood vessels of cats could be demonstrated when they were fed cholesterol and in addition were treated with (1) peptone or (2) histamine intravenously or fed (3) ammonium chloride.

HUMAN ATHEROSCLEROSIS IN RELATION TO THE CHOLESTEROL CONTENT OF THE BLOOD SERUM

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In this investigation we have attempted to determine whether or not there is any relationship between the degree of atherosclerosis in human arteries and the level of cholesterol in the blood serum.

There is a large literature concerning the relationship between spontaneous atherosclerosis of man and experimental atherosclerosis, which is easily obtained by feeding cholesterol to rabbits and in which the changes in the intima of the aorta and other vessels are very similar to those seen in human atherosclerosis. It has been shown that the experimental changes are dependent on hypercholesterolemia [1] and that if, for unknown reasons, no increase in the lipoids of the blood serum occurs there are also no deposits in the intima.

Some authors believe that hypercholesterolemia is a primary etiologic factor in spontaneous atherosclerosis of man as it appears to be in experimental atherosclerosis of rabbits. In considering this hypothesis account must be taken of the wide variation from person to person in the total cholesterol content of the serum. Values ranging from 131 to 392 mg. per hundred cubic centimeters were found for the cholesterol in the serum of healthy students and members of the staff of the College of Physicians and Surgeons,[2] and equally large differences were reported recently by Page, Kirk, Lewis, Thompson and van Slyke [3] for a group of healthy men. The concentration of no other constituent of the serum varies so much from person to person in health. The differences appear to be constitutional since in each of several persons the level of choles-

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1. Versé, M.: Beitr. z. path. Anat. u. z. allg. Path. **63**:789, 1916.

2. Sperry, W. M.: J. Biol. Chem. **114**:125, 1936.

3. Page, I. H.; Kirk, E.; Lewis, W. H., Jr.; Thompson, W. R., and Van Slyke, D. D.: J. Biol. Chem. **111**:613, 1935.

terol has been found to remain fairly constant over considerable periods (unpublished results). It is probable that each person maintains a particular level of cholesterol in the serum over long periods, perhaps throughout adult life, and it follows that a comparison of the concentration of cholesterol in the serum and the degree of atherosclerosis of the vessels in a sufficiently large number of subjects must decide whether there is any relationship between them.

Some attempts at such a comparison have been made in atherosclerotic patients, but it does not appear to us that this type of approach can yield conclusive results, because during life the disease is recognized only when the process has advanced so far as to cause secondary functional disturbances which in themselves may alter the serum cholesterol level. The beginning and mild forms of atherosclerosis, which would be of greatest value in studying the possible relationship to the concentration of cholesterol in the serum, present no symptoms which would bring the patients to the physician, and in any event the condition cannot be diagnosed in its early stages. Roentgenograms, which have been employed widely in the diagnosis of atherosclerosis of the peripheral vessels, usually show calcification of the media (Mönckeberg), which is considered by most investigators to represent an entirely different disease.

The degree of atherosclerosis can be established with certainty only by direct examination of the vessels, and therefore a comparison such as we wished to make was possible only on autopsy material. Unfortunately, the bodies which come to autopsy in a general pathologic laboratory are not suitable for this purpose since in most of them there is present not only the primary disease leading to death but also a terminal infection, either or both of which may have caused a marked change in the concentration of cholesterol in the blood serum. There is much evidence showing that the concentration of total cholesterol is lowered in the presence of infection, and recent results [2] indicate that the proportion of free cholesterol in the total cholesterol is increased under the same conditions.

In the present investigation we confined our attention to material from persons whose death had occurred suddenly, in most instances from trauma.[4] The copious material of the office of the Chief Medical Examiner of the City of New York offered an unusual opportunity for such a study. Cases were selected carefully according to criteria which are discussed in the section dealing with their selection. The investigation consisted in a comparison of the concentration of cholesterol in the blood serum with the lipid content of the intima of the aorta, which has been shown to parallel closely the degree of athero-

4. This approach was suggested by Dr. R. Schoenheimer.

sclerosis and to be practically uninfluenced by calcifying and scarring processes.[5] No definite relationship between the concentration of cholesterol in the serum and the degree of atherosclerosis was evident.

PREPARATION OF MATERIAL FOR ANALYSIS; CHEMICAL METHODS

At autopsy several cubic centimeters of blood, when possible unclotted blood from the heart, were transferred to a small bottle. The preparation and analysis of the aorta were carried out according to the method described by Schoenheimer [5a] and modified by Rosenthal.[5b] The whole aorta from a point immediately above the aortic valves to the bifurcation of the iliac vessels was dissected out, and the inner layer, containing the entire intima and a portion of the media, was stripped from the outer layer, containing the remaining media and the entire adventitia. The outer layer was discarded, as the inclusion of adventitial fat would vitiate the analytic results. The inner layer was transferred to a bottle, which was placed in the icebox along with the sample of blood until the following morning, when both were brought to the laboratory of the Babies Hospital for analysis.

The blood was centrifugated, and the serum was analyzed for total and free cholesterol by the method of Schoenheimer and Sperry.[6]

The lipid content of the aortic tissue was determined by chopping the tissue into small pieces, triturating thoroughly with anhydrous sodium sulfate and extracting with carbon tetra-chloride.[5b] An aliquot of the extract was taken to dryness, and the residue was weighed.[7]

SELECTION OF CASES

The greatest care was taken in the preliminary selection of cases for study and in the elimination of all in which there was evidence of abnormalities after the analyses had been completed. The criteria employed in the selection and exclusion of cases are discussed in the following section in detail, since it is evident that the conclusion reached in this investigation is valid only if the subjects were normal ante mortem and if the concentration of cholesterol in the serum did not change after death.

Analyses were made only when (1) death was sudden, (2) there was no gross evidence in any organ of pathologic change other than that associated with the fatal injury or atherosclerosis of the blood vessels and (3) autopsy was carried out soon after death. The reasons for the first and second criteria are discussed in the introduction. Even when death is caused by trauma, if the patient survives the injury by more than a few hours secondary hypostatic pneumonia, which may alter the cholesterol level of the serum, usually supervenes. The third requirement was necessary to insure minimum postmortem decomposition.

In all, 214 cases were selected for study on the basis of the foregoing criteria. Of these, 6 were lost through analytic error; 7 were discarded because there was insufficient serum for analysis. Fourteen samples of markedly hemolyzed serum

5. (*a*) Schoenheimer, R.: Ztschr. f. physiol. Chem. **160**:61, 1926; **177**:143, 1928. (*b*) Rosenthal, S. R.: Arch. Path. **18**:473 and 666, 1934.

6. Schoenheimer, R., and Sperry, W. M.: J. Biol. Chem. **106**:745, 1934.

7. The analyses were done with the technical assistance of Mr. Jack Graff and Mr. V. A. Stoyanoff.

were not analyzed, as it had become evident in the early part of the work that such samples consistently gave abnormal cholesterol values.

Forty of the 187 remaining cases were excluded on the basis of the microscopic examination of the tissues after the chemical analyses had been completed (leaving 147 cases). The presence of pneumonia, syphilis or other diseases, except atherosclerosis, was considered to be sufficient ground for ruling out the case.

Many cases also were excluded on the basis of abnormalities in the values for serum cholesterol. The success of the investigation depended on the assumption that no significant change in the concentration of cholesterol in the serum occurred post mortem. Practically no information on this point is available in the literature. Shope [8] noted the absence of cholesterol esters in blood serum taken post mortem from a bull which had died of tuberculosis, a calf which had died of an intestinal infection and a rabbit which had died of a broken neck. He concluded that a very active cholesterol ester-splitting enzyme is present in tissues.[9] Recent evidence [2] indicates, however, that the proportion of combined cholesterol in the total cholesterol of serum may be reduced markedly in the presence of infection, and it seems likely that the abnormal cholesterol picture observed by Shope in the serum of the bull and the calf was present before death and did not occur post mortem, as he assumed. The absence of combined cholesterol from the serum of a normal rabbit is more difficult to explain, but it is well known that the cholesterol metabolism of the rabbit is different from that of man. In this laboratory much larger fluctuations in the percentage of free cholesterol in the total cholesterol have been observed in rabbit serum than in human serum. Certainly it cannot be concluded from Shope's single observation that there is a rapid change in serum cholesterol following death.

Recently a new criterion has been established [2] for detecting variations from normal in the serum cholesterol. It has been found that the ratio between the serum cholesterol fractions in healthy persons is much more constant than has been recognized previously. In the serums from a large series of adults a minimum of 24.3 per cent and a maximum of 30.1 per cent of free cholesterol in the total cholesterol were found. Similar values were obtained for children beyond the neonatal period, and with few exceptions deviations from this small range were observed only in the presence of infection or hepatic disease. It does not follow necessarily that the finding of a normal ratio between the concentrations of total cholesterol and free cholesterol is definite proof that no change in the concentration of total cholesterol, in which we were primarily interested in the present study, took place after death; but it seems to us unlikely that a significant alteration would occur without being reflected in an abnormal ratio, unless the serum had been diluted or concentrated.

On the other hand, it also does not follow necessarily that the concentration of total cholesterol will be affected when there are variations from the normal proportion of free cholesterol in the total cholesterol. In Shope's rabbit the amount of total cholesterol was practically the same before and after death, although apparently a large change in the proportions of free and of combined cholesterol took place. Furthermore, the concentration of total cholesterol remains the same on incubation of serum in vitro, although there is a marked shift in

8. Shope, R. E.: J. Biol. Chem. **80**:127, 1928.

9. Shope presented further experimental evidence for this conclusion, which could not be confirmed by one of us (Sperry, W. M.: J. Biol. Chem. **113**:599, 1936).

the proportion (esterification[10]). Nevertheless, we felt that an abnormality in the relationship between total and free cholesterol offered presumptive evidence of some abnormality in the serum cholesterol picture and that it required the exclusion of the case (with some exceptions as noted below).

In 37 of the 147 cases remaining after the foregoing eliminations were made, the percentage of free cholesterol in the total cholesterol was above 30.1 per cent (the maximum found in healthy adults[11]). In 28 of these hemolysis was in all probability responsible for the increased proportion of free cholesterol. Since most, if not all, of the cholesterol of red blood cells is in the free form, such an effect would be expected when an appreciable degree of hemolysis had occurred. Nevertheless, as a large proportion of the samples showed hemolysis and as it is difficult to judge the extent of this process by inspection, we decided to analyze them in the hope that some at least might be saved. In adopting this course we relied on the percentage of free cholesterol in the total cholesterol to indicate whether there had been sufficient hemolysis to cause a significant alteration of the cholesterol content of the serum. Cases in which the percentage of free cholesterol in the total cholesterol was within normal limits were included even though some hemolysis had occurred.

Among the 37 cases in which there was an increased percentage of free cholesterol in the total cholesterol there were 13 (in 12 of which there was some hemolysis) in which the percentage was between 30.1 and 31.1 per cent. In view of this small deviation from normal it seems unlikely that the concentration of total cholesterol had been altered significantly, and these cases are included in our series provisionally (with a separate designation in the charts). There remained 24 cases which were excluded on the basis of an increased percentage of free cholesterol in the total cholesterol (leaving 123 cases). In 16 of these there was hemolysis; in 8 there was no evident explanation (infection, hepatic disease or hemolysis) for the abnormal proportion of cholesterol.[12]

In 13 cases the percentage of free cholesterol in the total cholesterol was below the minimum observed in normal living subjects (24.3 per cent), but in most cases it was only a little below (in 12 it was between 22 and 24.3 per cent). These abnormal results may be accounted for with high probability by the assumption that there had been an esterification in vitro. Such a change has been shown to occur[10] when blood serum stands at room temperature for a considerable period. Although, as pointed out, autopsy was performed as soon as possible after death, there was frequently a delay of a few hours during which some esterification might be expected. Through an oversight 2 samples (see the last footnote in table 2) stood for several hours at room temperature before analysis, and both showed a low percentage. There is no change in the concentration of total cholesterol during esterification,[10] and it seems justifiable, therefore, to include these 13 cases in

10. Sperry, W. M.: J. Biol. Chem. **111**:467, 1935.

11. In 26 of the 40 cases which were excluded on the basis of disease (infection in 22 and hepatic disease in 4) there was an increased percentage of free cholesterol in the total cholesterol. Thirteen of these serums were hemolyzed and were not included in the recently published summary of the data on the serum cholesterol.[2] In 14 cases the percentage of free cholesterol in the total cholesterol was not increased. These were excluded on the basis of pathologic pictures as follows: syphilis, 7; tumor of the brain, 2, and diphtheria, dystrophia adiposa genitalis, late pregnancy, tuberculosis and encephalitis, 1 each.

12. In view of the extreme conditions under which the samples were taken, the finding of a few unexplained abnormal values is not surprising.

our series. They are designated by separate symbols in the charts, as are the 13 cases in which there were slightly high percentages, for the benefit of any one who may not choose to accept them.

EXPERIMENTAL RECORDS

We summarize the disposition of cases in table 1. After the rejection of 91 cases for various reasons there remained 97 cases in which

TABLE 1.—*Disposition of 214 Cases*

Cases Excluded		Cases Retained	
Reason	Number	Reason	Number
Analytic error	6	With normal percentage of free cholesterol in total free cholesterol	97
Insufficient serum	7	With slightly increased percentage (some hemolysis in 12)	13
No analysis because of marked hemolysis	14	With decreased percentage	13
Microscopic evidence of disease	40*		
Increased percentage of free cholesterol in total cholesterol:			
With hemolysis	16		
Without hemolysis	8		
Totals	91		123

* See footnote 11.

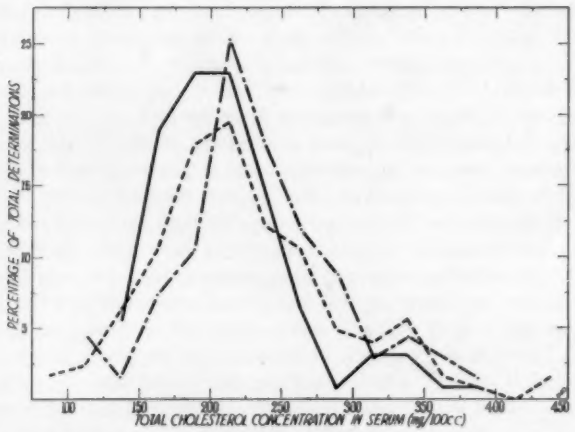

Chart 1.—Frequency distribution of total cholesterol concentrations. The solid line indicates the data obtained by Sperry;[2] the dot and dash line, the data obtained by Page, Kirk, Lewis, Thompson and Van Slyke,[3] and the dash line, the data obtained in the present investigation.

the percentage of free cholesterol in the total cholesterol was within normal limits, 13 in which the percentage was slightly elevated (in all but 1 in the presence of hemolysis) and 13 in which it was reduced (in all probability because of esterification)—a total of 123 cases.

In chart 1 we compare the frequency distribution (calculated on a percentage basis) of the concentrations of total cholesterol in our

selected cases with those which Page and his colleagues [3] obtained in a study of healthy men throughout the life span and with the findings reported by one of us [2] in young healthy adults. The comparison offers strong support for our assumption that no significant change in the antemortem constitutional level of total cholesterol occurred after death.

Cause of Death.—In a large majority of the cases, as shown in table 2, death was due to trauma (in most instances from shooting, stabbing or a motor car accident). The diagnosis of alcohol poisoning was made only after microscopic examination of the tissues had established the absence of any other pathologic changes which might account for death and after the brain and liver had been submitted to chemical examination in the toxicologic laboratory of the office of the Chief Medical Examiner. A positive test for alcohol was obtained in a large proportion of the cases, but death was not ascribed to alcohol unless there was no other evident cause.

Macroscopic Atherosclerosis.—The degree of atherosclerosis as evident from gross examination of the aorta is given in table 2 in the form of numbers, 0 indicating absence of atherosclerotic lesions, 1 a few isolated early lesions, and so on up to 6, which represents the most advanced stage of the disease, with ulcerative abscesses and severe atherosclerotic changes in the intima and media.

Physical Measurements, etc.—Aside from the data given in table 2, records were kept of the race, sex, body length and weight, changes in blood vessels other than the aorta (especially the coronary artery and the arteries of the brain), the thickness of the wall of the left ventricle, weight of the heart and so forth. There was no correlation between any of them and the amount of cholesterol in the blood serum. The finding in regard to the weight of the heart is in accord with the recent report of Page, Kirk and Van Slyke,[13] who observed no relationship between the concentration of cholesterol in the serum and the blood pressure in living subjects.

COMMENT

The findings of this investigation lead to the conclusion that there is no correlation between the concentration of cholesterol in the blood serum and the lipid content of the aorta. This is shown clearly by charts 2 to 6, in which the two quantities are plotted against each other. The points are scattered at random in all of the age groups. Omission of the cases in which there was a slightly abnormal cholesterol ratio does not alter the foreging conclusion. Since it has been demonstrated

13. Page, I. H.; Kirk, E., and Van Slyke, D. D.: J. Clin. Investigation **15**:109, 1936.

TABLE 2.—*A Comparison of the Total Cholesterol Content of the Blood Serum with the Degree of Atherosclerosis*

Cause of Death	Degree of Athero-sclerosis (Macroscopic)	Free Cholesterol in Total Cholesterol, per Cent	Total Cholesterol in Serum, Mg. per 100 Cc.	Lipid in Aorta, Mg.
Age, 11 to 29 years				
Trauma	0	29.7	283	24
Trauma	0	28.9	205	40
Alcoholism	0	27.0	282	90
Trauma	2	24.9	199	272
Trauma	0	23.8*	163	78
Cerebral hemorrhage	2	28.0	235	126
Trauma	1	22.7*	229	148
Trauma	1	28.7	173	126
Trauma	0	30.2†	222	115
Trauma	0	27.5	180	209
Trauma	1	23.1*	446	175
Trauma	0	23.0*	204	92
Trauma	0	26.2	253	95
Alcoholism	0	29.2	282	82
Unknown	1	26.0	188	132
Trauma	2	30.0	215	333
Trauma	1	27.0	226	242
Trauma	1	24.7	158	216
Alcoholism	2	26.8	439	112
Trauma	0	24.9	183	44
Trauma	1	29.2	82	119
Age, 30 to 39 years				
Trauma	1	25.2	182	96
Alcoholism	1	27.0	247	132
Trauma	1	29.4	265	129
Trauma	0	30.5†	200	147
Trauma	1	25.3	152	102
Trauma	2	30.7†	227	413
Alcoholism	1	24.2*	188	92
Trauma	1	27.2	173	154
Trauma	2	28.1	153	295
Trauma	2	26.0	131	417
Poisoning	1	28.6	250	173
Trauma	2	26.7	164	186
Alcoholism	1	25.4	134	145
Unknown	1	28.3	331	99
Trauma	4	19.1*	188	103
Poisoning	1	28.7	228	173
Trauma	1	28.8	168	141
Alcoholism	1	24.3	324	129
Trauma	1	27.5	178	110
Trauma	2	22.2*	225	322
Trauma	1	24.8	339	294
Trauma	2	31.1†	250	195
Trauma	2	30.3†	211	435
Trauma	1	30.6†	242	103
Unknown	0	27.9	219	104
Trauma	2	28.0	159	198
Trauma	2	27.2	176	176
Trauma	3	23.5*‡	250	495
Coronary occlusion	1	28.8	328	276
Trauma	3	29.6	120	434
Alcoholism	2	27.6	335	188
Unknown	1	29.0	213	154
Poisoning	2	27.2	216	210
Unknown	1	25.3	223	159
Trauma	1	25.8	268	241
Cerebral hemorrhage	4	27.7	249	1,125

* The percentage of free cholesterol in the total cholesterol was below the normal range (see text).

† The percentage of free cholesterol in the total cholesterol was above the normal range (see text).

‡ Through an oversight the sample of serum stood for several hours at room temperature before analysis.

TABLE 2.—*A Comparison of the Total Cholesterol Content of the Blood Serum with the Degree of Atherosclerosis—Continued*

Cause of Death	Degree of Athero-sclerosis (Macroscopic)	Free Cholesterol in Total Cholesterol, per Cent	Total Cholesterol in Serum, Mg. per 100 Cc.	Lipid in Aorta, Mg.
Age, 40 to 49 years				
Trauma	1	24.7	202	161
Trauma	2	27.1	188	258
Coronary occlusion	2	27.2	217	624
Trauma	2	26.2	149	122
Trauma	2	30.8†	349	168
Alcoholism	0	27.9	136	117
Trauma	3	23.6*	132	125
Coronary occlusion	3	24.3	312	562
Trauma	3	25.9	198	889
Trauma	2	30.9†	195	240
Trauma	1	28.2	222	161
Trauma	2	24.8	206	218
Trauma	2	25.1	211	258
Trauma	2	24.8	218	287
Trauma	3	28.3	193	897
Trauma	2	26.7	346	403
Alcoholism	2	26.3	255	276
Trauma	2	28.0	159	259
Trauma	3	29.3	222	809
Trauma	2	30.4†	362	412
Unknown	4	23.8*	335	370
Alcoholism	2	26.2	247	259
Coronary occlusion	4	31.0†	242	849
Trauma	3	24.9	319	395
Atherosclerosis	3	27.2	362	1,177
Cerebral hemorrhage	3	28.1	258	1,140
Age, 50 to 59 years				
Alcoholism	4	24.3	217	876
Trauma	1	31.0†	174	528
Trauma	6	28.6	179	1,303
Trauma	4	29.6	205	498
Trauma	2	30.4†	123	446
Cerebral hemorrhage	1	28.3	139	489
Trauma	3	29.0	127	427
Trauma	2	25.1	199	760
Alcoholism	2	29.2	139	692
Alcoholism	4	26.9	247	756
Trauma	6	28.3	176	1,327
Trauma	3	28.6	241	526
Trauma	2	27.9	292	202
Atherosclerosis	3	29.9	187	608
Trauma	2	30.0	215	333
Trauma	4	29.6	183	1,745
Trauma	4	30.0	215	1,412
Alcoholism	4	26.1	206	584
Trauma	3	30.2†	217	946
Trauma	3	27.6	273	438
Trauma	2	23.6*	267	404
Trauma	2	24.1*	195	893
Trauma	3	23.5*‡	251	334
Age, 60 to 80 years				
Trauma	3	27.1	288	294
Coronary occlusion	2	25.2	302	510
Trauma	3	24.5	249	221
Lesion of mitral valve	3	27.3	271	302
Trauma	3	28.2	227	590
Unknown	2	27.0	97	443
Trauma	4	29.8	182	1,658
Trauma	4	28.8	186	774
Alcoholism	3	27.1	294	441
Trauma	4	27.5	218	1,406
Trauma	4	28.8	160	2,575
Coronary occlusion	6	24.9	272	3,666
Trauma	4	28.5	195	565
Trauma	3	27.8	378	1,564
Trauma	6	26.4	170	2,673
Trauma	5	28.8	118	1,970
Coronary occlusion	5	26.7	311	2,177

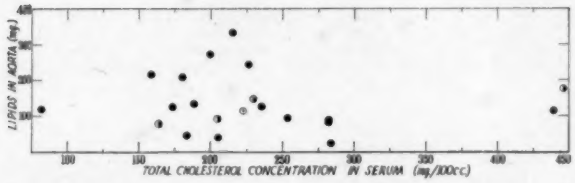

Chart 2.—Comparison of the concentration of total cholesterol in the blood serum with the lipid content of the aorta of persons from 11 to 29 years of age. In charts 2 to 6 the black circle represents the cases in which the percentage of free cholesterol in the total cholesterol was normal; the dotted circle, the cases in which the percentage of free cholesterol in the total cholesterol was above normal (see text), and the black and white circle, the cases in which the percentage of free cholesterol in the total cholesterol was below normal (see text).

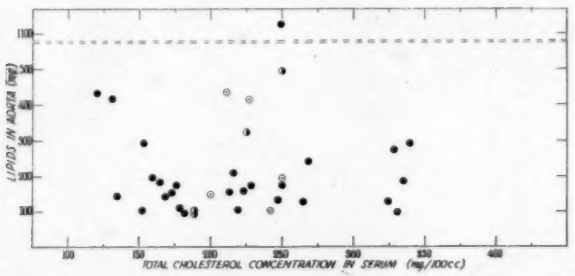

Chart 3.—Data for persons from 30 to 39 years of age.

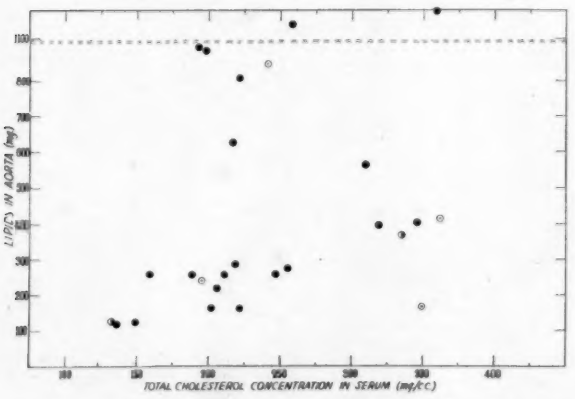

Chart. 4.—Data for persons from 40 to 49 years of age.

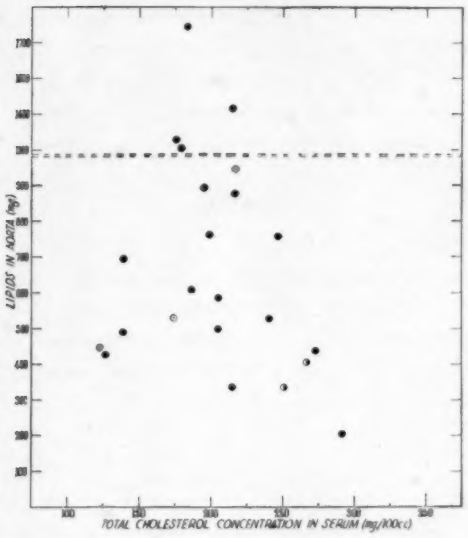

Chart 5.—Data for persons from 50 to 59 years of age.

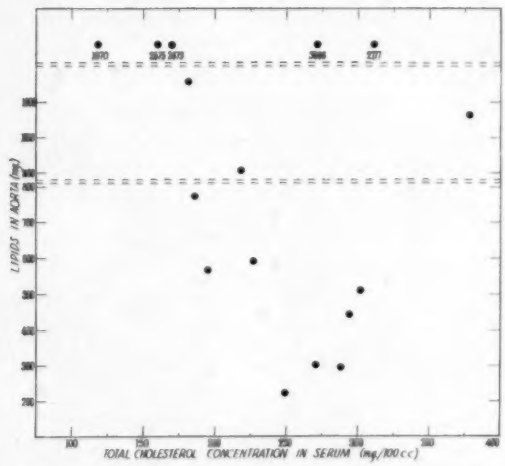

Chart 6.—Data for persons from 60 to 80 years of age.

by Schoenheimer [5a] and Rosenthal [5b] that there is a parallelism between the lipid content of the aorta and the degree of atherosclerosis [14] and since it has been shown in the present investigation that there is no correlation between the lipid content of the aorta and the cholesterol content of the blood serum, it follows that there is no direct relationship between the concentration of cholesterol in the blood serum and the degree of atherosclerosis in the aorta.

SUMMARY

The concentration of cholesterol in the blood serum was compared with the degree of atherosclerosis in the aorta, as measured by the lipid content, in 123 healthy persons who had died suddenly from violence. No relationship was evident, and it is concluded that the incidence and severity of atherosclerosis are not directly affected by the level of cholesterol in the blood serum per se.

14. The findings of Schoenheimer and Rosenthal are confirmed in the present study. A comparison of the degree of atherosclerosis as determined macroscopically with the lipid content of the aorta shows a general parallelism (table 2). There are a number of apparent exceptions which are to be expected occasionally in view of the difficulty in classifying aortas according to the extent and severity of the atherosclerosis noted on macroscopic examination (see the article by Rosenthal [5b] for a detailed discussion). There is no correlation between the macroscopic rating of the degree of atherosclerosis and the cholesterol content of the blood serum.

VASCULARIZATION AND HEMORRHAGE OF THE INTIMA OF ARTERIOSCLEROTIC CORONARY ARTERIES

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TORONTO, CANADA

This study was suggested by the finding of varying degrees of hemorrhage within the intima in the coronary arteries in nine consecutive cases of recent coronary thrombosis. In some of these cases, numerous capillary channels were seen in the inner layers of the intima in close proximity to the extravasated blood. Serial section showed one of these capillaries to arise, not from the vasa vasorum, but from the lumen of the partially occluded vessel. In the light of this finding it was felt that rupture of discrete intimal capillaries might be the cause of the hemorrhage previously noted, and further, that intimal hemorrhage might be concerned with the mechanism of coronary thrombosis.

The literature on the vascularization of the coronary arteries is confined almost exclusively to reports on abnormal proliferation of the vasa vasorum. With well established atherosclerosis the vasa vasorum have been described as penetrating the media to reach the intima.[1] However, a discrete capillary circulation of the intima, distinct from the vasa vasorum, has been observed twice in arteriosclerotic arteries of man. Robertson [2] found minute nutrient vessels arising from the lumen of the ascending aorta in two cases. These vessels penetrated the intima and branched in the inner third of the media. Leary [3] described proliferating intimal capillaries in four of twenty-one cases of coronary thrombosis, and in at least one of these serial section revealed a discrete opening of a capillary into the lumen. Leary made no comment on this finding; in fact, in a later paper [4] he doubted the existence of a true blood supply to atherosclerotic plaques. The association of intimal hemorrhage with these discrete capillaries has not been described previously, although hemorrhage alone has been noted in the intima in thrombosed coronary arteries. Boyd [5] found hemorrhage

Department of Pathology, University of Toronto.

1. Gross, L.; Epstein, E. Z., and Kugel, M. A.: Am. J. Path. **10**:253, 1934. Klotz, Oskar: Arteriosclerosis: Diseases of the Media and Their Relation to Aneurysm, Lancaster, Pa., New Era Printing Co., 1911, p. 13.
2. Robertson, H. F.: Arch. Path. **8**:881, 1929.
3. Leary, T.: Arch. Path. **17**:453, 1934.
4. Leary, T.: Am. Heart J. **10**:328, 1935.
5. Boyd, A. N.: Am. J. Path. **4**:159, 1928.

in the intima in two cases, but he regarded it as a part of an acute inflammatory process, the hemorrhage being derived from the usual vasa vasorum. Leary [3] also noted intimal hemorrhage in association with coronary thrombosis, but he interpreted it as secondary to a rupture of the intima from the lumen into an atheromatous focus.

This paper deals with the extent of the vascularization of the intima of the coronary artery and with the mechanism of production and the structure of intimal hemorrhage. The possible relation of intimal hemorrhage to coronary thrombosis is briefly discussed.

MATERIAL AND METHOD

The material was obtained at autopsies performed on children and adults in this department and in the Sick Children's Hospital, Toronto, over a period of approximately six months. The series included twelve children, eleven adults under the age of 40, and thirty-three older persons. Included in the last group were sixteen consecutive patients with coronary thrombosis, in nine of whom the thrombosis was recent while in the remaining seven the thrombi showed evidence of partial or complete organization. In the children and in the adults under the age of 40, blocks were taken from the proximal 1 cm. of the left anterior descending coronary artery. In the older persons, blocks were taken through the centers of atherosclerotic plaques in any of the coronary branches, through areas of visible hemorrhage in the intima and when thrombosis was present through various parts of the occluded vessel. In some of the blocks which showed proliferating capillaries in the inner third of the intima, serial sections were made in an attempt to demonstrate the opening of the capillaries into the lumen. Serial sections were cut at 8 to 10 microns, and every section for a variable distance was mounted and stained.

OBSERVATIONS

No evidence of vascularization of the intima was found in the coronary arteries of the children. In the coronary arteries of the young adults, thickened, hyaline, endarteritic plaques were not infrequently observed, but in none of these was there any evidence of a distinct intimal blood supply. Dilated capillary channels were occasionally seen in the outer third of the intima, but these, from their position, were regarded as penetrating branches of the vasa vasorum. In the older persons, coronary arteries affected by atherosclerosis but without thrombosis not infrequently showed proliferating capillaries in the inner third of the intima, internal to the site of an atheroma and sometimes just beneath the endothelium. In almost every coronary artery occluded by a thrombus, either recent or organized, an atherosclerotic thickening of the intima was a marked feature. Proliferating intimal capillaries lying close to the lumen were seen at the site of thrombosis in fifteen of the sixteen thrombosed coronary arteries. Intimal capillaries were most numerous in those which showed advanced organization of the occluding thrombus. The capillaries did not remain confined to the region of the former lumen, however, but penetrated the depths of

the intima to lie in close contact with the atheromatous plaques. Intimal capillaries were also numerous in association with recent thrombi, and in all but one of the arteries in which this was shown no definite evidence of previous thrombosis could be seen.

Serial section was done in eleven coronary arteries in an attempt to demonstrate the point of origin of the intimal capillaries. In four, the capillaries were found to arise by discrete openings from the lumen (fig. 1). In the remaining ones the capillaries could be traced to a point just beneath the endothelium, but there they faded out and disappeared. In some of the unsuccessful serial sections several sections had been lost in the process of cutting, and this may explain the failure to demonstrate the exact point of origin of the capillaries. In others, the capillaries were found to run parallel to the lumen for the entire thickness of the paraffin block. In each of the blocks in which the search was unsuccessful, however, the capillaries remained confined to the vicinity of the lumen; they lay internal to or ramified within atheromatous foci, the outer parts of which appeared to separate them from the terminal branches of the vasa vasorum. From their position, therefore, and from the evidence obtained from the serial sections in which the origin of such capillaries from the lumen was observed, all capillaries which were seen in the inner third of the intima were regarded as arising from the lumen. The four successful serial sections were done in coronary arteries showing atherosclerosis without thrombosis (one), recent coronary thrombosis (one) and organized coronary thrombosis (two). In the two arteries which showed organized thrombi the intimal capillaries appeared to arise from recanalized channels and then to penetrate the intima in close proximity to atheromatous foci. In the other two arteries no evidence of former thrombosis was found (fig. 1).

Intimal hemorrhage of coronary arteries has been observed in two types of vessels: (1) in arteries affected by atherosclerosis but without evidence of thrombosis [6] (nine) and (2) in recently occluded coronary vessels at the site of occlusion (nine). The hemorrhages in the two types of vessels were similar in several ways, but to avoid confusion they will be described separately.

The gross appearance of the nine discrete intimal hemorrhages observed in arteries unaffected by thrombosis was characteristic. On opening the lumen of the vessel the hemorrhage appeared as an oval

6. The nine discrete intimal hemorrhages were found in two classes of patients: four in whom no evidence of thrombosis was found in any of the coronary branches; five in whom a thrombus was found in one of the coronary branches, but it was located at a point well removed from the site of the discrete hemorrhage. For example, in one person the left anterior descending branch was occluded by a recent thrombus, but the discrete hemorrhage was found in the right coronary artery. The latter vessel was patent throughout its entire length.

or rounded area of dark red or brown discoloration of the tissues immediately underlying the endothelium. The hemorrhages measured up to 5 mm. in diameter. Each had occurred into an atheromatous

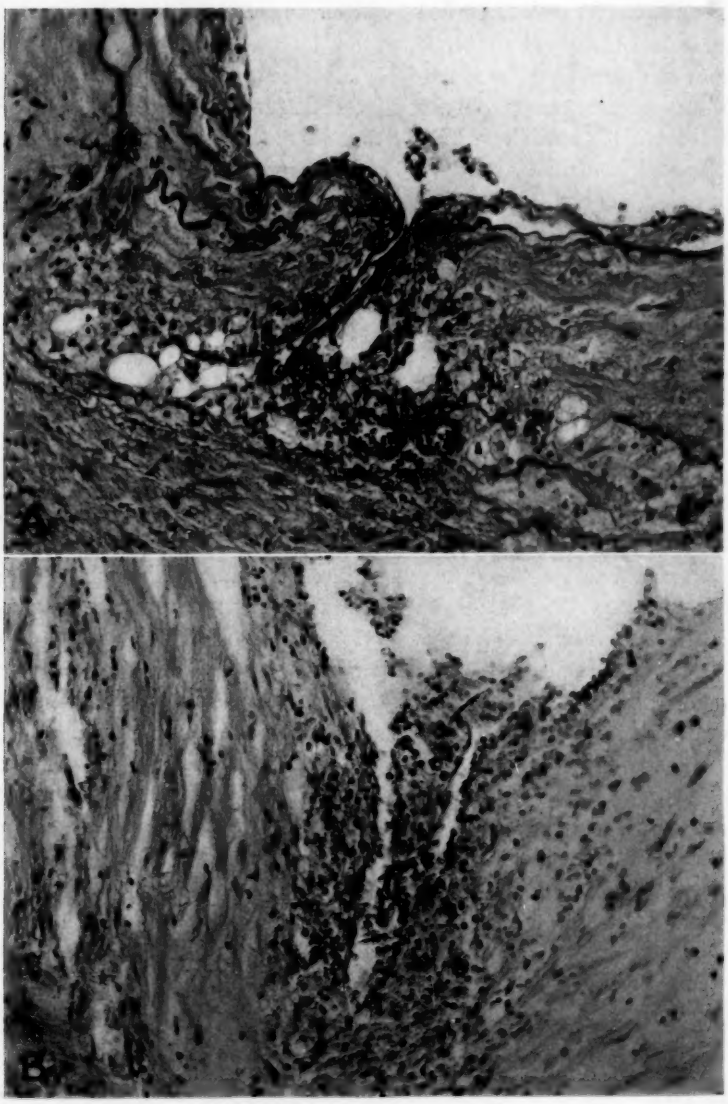

Fig. 1.—*A* shows the origin of a discrete capillary from the lumen of an artery affected by atherosclerosis but without thrombosis. The section is stained with a modified Weigert and hematoxylin-eosin stain; × 160. *B* shows the origin of intimal capillaries from the partially occluded lumen of a coronary artery affected by recent thrombosis; × 160.

plaque. The overlying endothelium was intact; as a rule it was elevated to form a small hummock distinct from the wider swelling of the atheromatous plaque proper, but occasionally it was depressed and dimpled. The cut surface of the hemorrhagic area was reddish brown. In some of the hemorrhages the contents were quite liquid, the incised surface presenting an irregular slitlike defect resembling a small dissecting aneurysm of the intima. The tissues underlying the hemorrhage always presented a yellowish-gray, softened appearance, like that of atheroma. The hemorrhage tended to track irregularly into this atheromatous material.

Microscopically, each of the discrete hemorrhages had occurred into a part of the intima affected by lipoid degeneration associated with softening of the tissues as found in atheroma. The softening process was a marked feature in all these arteries but one. Arteries in which the hemorrhage was obviously recent showed numbers of capillary channels in the inner layers of the intima in close proximity to the extravasated blood. In one instance in particular (fig. 2 *A*) several dilated, engorged capillaries were lying close to the inner surface and spreading outward into an adjacent atheromatous plaque. At the point of entrance into the softened plaque the capillary walls could not be made out and a localized mass of hemorrhage was found. Capillaries such as these, from their position and from evidence obtained from serial sections, were considered to arise by discrete openings from the lumen. When the hemorrhage was older the capillaries were usually difficult to find, but when organization of the extravasated blood had begun numerous young capillaries appeared. Microscopically, the nine discrete hemorrhages varied principally in the age and amount of the extravasated blood and in the local reaction of the tissues to its presence. Some represented the stage of capillary dilatation and rupture with hemorrhage into the intima; others, the stage of hemosiderin formation and the repair of the lesion by granulation tissue. The endothelium overlying each hemorrhage was intact, and no evidence of beginning thrombus formation was noted. In only one was there any indication of necrosis of the adjacent intimal tissue. In seven chronic inflammatory infiltration, consisting principally of lymphocytes, was seen in the vicinity of the hemorrhage, but it was almost entirely absent in two. It tended to be more intense when organization of the hemorrhage was advanced, but this was not always the rule.

Hemorrhage into the intima, similar in many respects to the discrete hemorrhages just described, has been observed in association with recent coronary thrombosis. The structure of this hemorrhage will be discussed under microscopic observations. In the course of 249 routine autopsies in the six months' period, sixteen cases of coronary thrombosis were encountered. Nine of these were recent while in the remain-

der the thrombi showed evidence of partial or complete organization. In one of the nine recent cases, both the left anterior descending and the right coronary artery were occluded by recent thrombi; ten recently

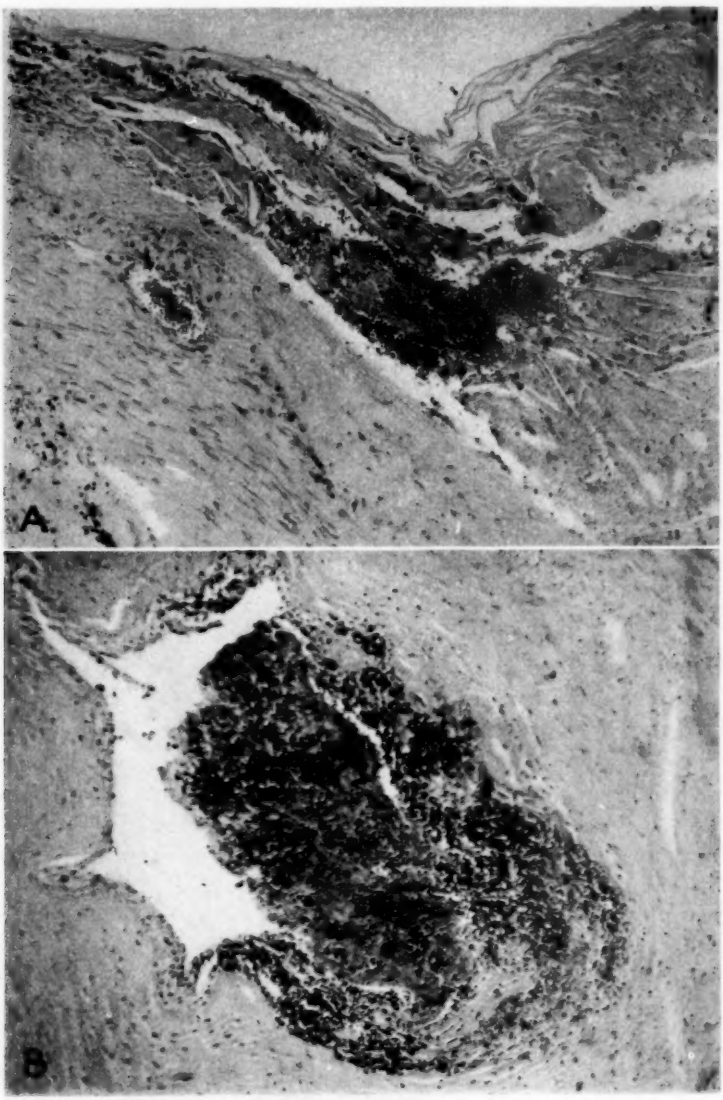

Fig. 2.—*A* shows several dilated capillaries close to the lumen of an artery which is not affected by thrombosis. A mass of hemorrhage is seen at the point of entrance of the capillaries into an atheromatous focus; × 80. *B* shows the elevation of the endothelium by the hemorrhage at the site of attachment of a thrombus; × 80.

thrombosed vessels have therefore been studied. Each of these ten vessels was affected by arteriosclerosis. A discrete atheromatous plaque lay in intimate association with the occluding thrombus in nine of the ten arteries. The plaque was proximal to the thrombus in four, and it projected into the approximate center of the thrombus in the remaining five. In the latter five it was not possible to tell whether the portion of thrombus lying proximal to the stenosing arteriosclerotic plaque was propagated or not. In two of the arteries the thrombus involved the immediate origin of the artery and protruded from it in a nipple-like manner. In addition to the presence of the stenosing plaques mentioned, the lumen of each thrombosed vessel was narrowed by a diffuse thickening of the intima.

Microscopically, the recently thrombosed vessels presented certain features in common. In eight of the ten arteries there was marked lipoid degeneration with deposition of cholesterol in the necrosed area of the intima. Atheroma was present in the youngest of the series (41 years) and in the oldest (82 years). As far as this series is concerned it appears to be a characteristic lesion. The two exceptions deserve some comment. In one, the recent thrombus was found in a part of the artery which showed evidence of former thrombosis and organization in relation to chronic endarteritis. In the other, the intima was everywhere dense and hyaline except at the point of attachment of the thrombus, where an irregular defect filled with blood was found. Although atheroma was such a marked feature in the series, calcification was conspicuous by its absence. It was sometimes present in other branches of the coronary system, but only once was it found in any quantity at the site of thrombosis. This was in the only block of tissue which could not be readily sectioned without decalcification. In this one, however, the calcification was localized to the outer layers of the intima, the portions in close relation to the lumen being softened and degenerated. A striking example of the relative absence of calcification at the site of thrombosis was provided by a case which has been observed since the conclusion of the series described. In this case, the three main coronary arteries were intensely calcified; they were so hard that it was impossible to section them with the knife or scissors without causing fragmentation of the walls of the vessels. This condition of calcification was general except for the immediate points of origin of the two main trunks and a distally placed small segment of the right coronary artery. In the latter area the wall of the vessel could be incised cleanly and with ease, and there the thrombus was found.

Each of the ten recently thrombosed arteries showed some degree of hemorrhage into the softened intimal tissue. It varied considerably in amount; in most of the arteries it was moderate, in a few it was marked, and in only one was it so slight as almost to escape notice.

The portion of the intima affected was sometimes quite small; in some arteries repeated blocks had to be cut before the hemorrhage was finally found. Its position in relation to the occluded lumen is shown diagrammatically in figure 3. In seven of the arteries (*1, 2, 3, 4, 5b, 6* and *7*) it was found in close association with the site of attachment of the thrombus. In two (*8* and *9*) it lay deep in the substance of an atherom-

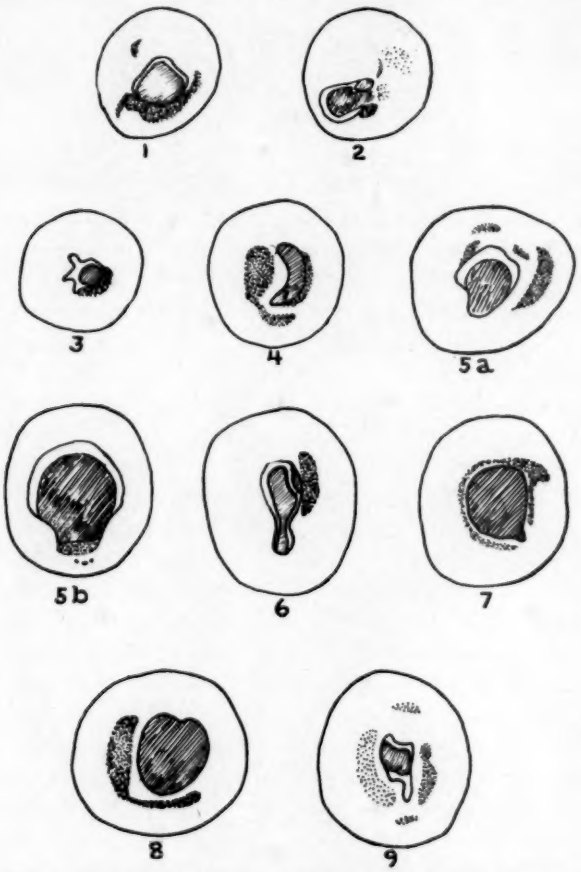

Fig. 3.—The drawings show the relation of the intimal hemorrhages to the points of attachment of the thrombi in the ten recently thrombosed coronary arteries. The dotted areas represent the intimal hemorrhages while the diagonally shaded areas represent the thrombi.

atous plaque on the side of the lumen to which the thrombus was attached. In one (*5a*) no apparent relation to the thrombus (as determined in the sections) could be found. The coronary arteries showing thrombosis of an older nature were examined for evidence of intimal

hemorrhage, and in several of them collections of hemosiderin were found in the deeper layers of the intima, well away from the region of the former lumen. These collections of blood pigment were interpreted as being the result of hemorrhage into the wall of the vessel at the time of the original thrombosis and subsequent transport of the pigment by macrophages.

In fifteen of the sixteen arteries showing coronary thrombosis, both old and recent, capillaries were found in the inner layers of the intima. In some they lay in close proximity to the extravasated blood. Serial section of three of these vessels showed the capillaries to arise from the lumen of the occluded artery. In the remaining arteries the capillaries, because of their proximity to the lumen, were assumed to arise in a similar manner.

COMMENT

Vascularization of the intima appears to be a process which is confined almost exclusively to coronary arteries affected by atherosclerosis either with or without thrombosis. It has not been seen in children nor has it been seen in young adults, even when nodular endarteritis was present. It apparently develops in one of the following ways. (1) in response to the demands for nutrition by the plaques of endarteritis; (2) in secondary relation to partial thrombosis. The thrombus may be small but sufficient to stimulate proliferation of capillaries (granulation tissue) which later extend into the intima.

Regardless, however, of the mechanism of vascularization, capillaries have been seen to penetrate the intima and to ramify within the endarteritic and atheromatous plaques or in the tissues neighboring thereto. Their presence is sometimes associated with evidence of hemorrhage into the adjacent tissue, and from the microscopic observations there is reason to believe that the hemorrhage results directly from rupture of the capillary walls.

The integrity of a capillary wall in any part of the body depends on (1) the pressure of blood within the lumen, (2) the strength and elasticity of the wall and (3) the rigidity of the supporting stroma. Intracapillary pressure appears to have an important but subsidiary bearing on the etiology of hemorrhage into the intima of coronary arteries. The discrete capillaries just described are peculiar in that they are in direct communication with the lumen of a large artery in which the pressure of blood, even if normal, is relatively high, closely approximating that in the ascending aorta.[7] They are not, like other capillaries, at the end of a long series of arteries and arterioles which absorb much

7. Klotz, Oskar, and Lloyd, Wray: Tr. A. Am. Physicians **45**:108, 1930.

of the pressure by friction. In this regard, Landis,[8] by cannulation of capillaries in the skin, found that the pressure in various parts of capillary loops in normal persons varied between 6 and 48 mm. of mercury, i. e., less than half the normal systolic pressure in the ascending aorta. Clark [9] found that young capillaries in the rabbit's ear, as observed through a transparent chamber, were readily dilated and ruptured by slight external pressure or by increased blood pressure. With hypertension the strain on the walls of capillaries in the intima of coronary arteries should be even greater. An analysis of the data on patients with discrete intimal hemorrhages without thrombosis showed the blood pressure shortly before death to have been in excess of 150 systolic and 110 diastolic in five. In the remaining patients it was at a normal level. Persistent hypertension, therefore, although a common feature in patients with intimal hemorrhage, is not always found. It is reasonable to suppose, however, that if it is present it may be a predisposing cause of capillary rupture. Cutter and Marquardt,[10] on exerting negative external pressure on the capillaries of the skin, found that rupture occurred at lower negative pressures in persons with hypertension (200 mm. plus) than in those with normal pressures.

Variation in the strength of capillary walls probably plays little part in the etiology of hemorrhage into the intima of coronary arteries. The normal ability of endothelial cells to contract and dilate may be affected by inflammatory conditions in their immediate neighborhood. Inflammatory infiltration in association with intimal hemorrhage, with or without thrombosis, was noted in most of the arteries that showed such hemorrhage, but it was almost entirely absent in a few. When present, no cuffing of the capillaries was seen, and the lining endothelial cells appeared healthy. Inflammatory infiltration appeared in general to be related to the disintegration of red cells or to necrosis of the adjacent tissue and was probably secondary. However, as each of the hemorrhages in this series had occurred in a middle-aged or elderly person the influence of age on the elasticity of capillary walls must not be completely ignored. In this regard Cutter and Marquardt [10] noted an increase in fragility of capillary walls in direct proportion to the age of the patient.

The third factor, the rigidity of the supporting stroma, probably plays the most important part in preserving the continuity of the wall of the intimal capillary. The most striking observation in this study

8. Landis, E. M., quoted by Wright, I. S., and Duryee, A. W.: Ann. Int. Med. **52**:545, 1933.

9. Clark, E. R.: Ann. Int. Med. **9**:1043, 1936.

10. Cutter, I. S., and Marquardt, G. H.: Proc. Soc. Exper. Biol. & Med. **28**:113, 1930.

was that intimal hemorrhages, when recent, had occurred almost without exception into softened plaques. The degree of atheroma was usually pronounced, the evidence of lipoid degeneration with or without deposition of cholesterol crystals, being marked. Capillaries, when present, were often greatly dilated in the vicinity of the softened area, but in regions where they traversed dense hyaline tissue their lumens were small (fig. 1 *A*). The softening process appears to allow the capillary wall to dilate as a result of the pressure within its lumen. The actual rupture probably occurs from overdilatation and from increased fragility due to advancing age.

The relation of intimal hemorrhage to the mechanism of coronary thrombosis is problematic. Evidence in favor of its primary importance in initiating the deposition of thrombi is as yet mostly circumstantial. The finding of discrete intimal capillaries, atheroma and hemorrhage in practically every coronary artery showing recent thrombosis in this series suggests that these factors represent a chain of events which lead to thrombosis. Atheroma, resulting in loss of rigidity of the intercapillary stroma, is a characteristic lesion in persons between 45 and 65 years of age, and at this age period coronary thrombosis has been commonly encountered. Persons younger or older than this commonly present either nodular endarteritis or calcification of their arteries, conditions which, if capillaries were present, would tend to maintain a more rigid intercapillary stroma. However, only a small amount of direct evidence has been obtained indicating that hemorrhage into the intima is the immediate cause of thrombosis. In several of the thrombosed arteries, but not in all, the thrombus was attached to the wall at a point in close proximity to the extravasated blood. It is conceivable that the hemorrhage may have damaged the endothelium sufficiently to enhance thrombosis.

The fact that intimal hemorrhage has been noted in some of the arteries in this series without definite evidence of thrombosis of the adjacent lumen deserves some comment. In some of the discrete hemorrhages the amount of extravasated blood was actually greater than that found in the intima of most of the coronary arteries showing recent thrombosis. In a few instances the discrete intimal hemorrhage appeared to be sufficiently marked to have precipitated thrombosis if it had been the only factor necessary. It seems probable, therefore, that other factors play some part in the process. Of these, the eddying or stagnation of blood due to deformity of the lumen should be considered. Stenosis of the coronary arteries by atherosclerotic plaques with resulting alteration in the direction and speed of the blood flow has long been held as a cause of thrombosis. Stenosing atherosclerotic plaques were found at the site of thrombosis in nine of the ten coronary arteries

showing recent thrombosis in this series. However, if alteration in blood flow from stenosis were the only factor necessary, coronary thrombosis would be a commoner lesion than it actually is. It seems probable, therefore, that damage to the endothelium at a vulnerable point is also needed. It is suggested that intimal hemorrhage resulting from the rupture of discrete intimal capillaries may produce such damage.

SUMMARY

Vascularization of the intima of coronary arteries by discrete capillaries which arise from their lumens is a common finding in association with atherosclerosis. It is particularly marked in thrombosed coronary arteries. It has not been found in normal coronary arteries or in those affected by early nodular endarteritis.

Hemorrhagic lesions within the intima, which had occurred into atheromatous foci, have been observed in a number of arteriosclerotic coronary arteries, including those from nine consecutive patients with recent coronary thrombosis. When the hemorrhage was recent, capillary channels were found in the inner layers of the intima in close proximity to the extravasated blood. The sequence of events in the production of intimal hemorrhage appears to be (1) endarteritis with vascularization of the intima, (2) atheroma with resultant softening of the intercapillary stroma and (3) capillary rupture. Because discrete intimal capillaries, atheroma and intimal hemorrhage have been found in practically every coronary artery showing recent thrombosis in this series it is suggested that these factors represent a chain of events which lead to thrombosis, the immediate cause being damage to the endothelium by the extravasation of blood into the intima.

SHOCK

A DEFINITION AND DIFFERENTIATION

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Shock has been confused with hemorrhage, with cardiac and vasomotor deficiencies, with neurologic disturbances, with fat embolism, with dysfunction of the sympathico-adrenal system and even with hyperoxemia. Few authors have attempted to define the condition that they discussed, and there has been no agreement on a comprehensive definition. Confusion between conditions having some features in common will persist until some definite entity can be segregated from the heterogeneous group. During the World War the Medical Research Council of Great Britain appointed a special commission to make a coordinated study of all phases of shock.[1] Serving on this commission were Englishmen and Americans of distinction in various fields of medical science. Casualities in field hospitals and clearing stations provided a wealth of material, including extensive injuries not involving vital structures and not accompanied by serious hemorrhage. Investigations lasting more than a year were made on wounded soldiers and on shock produced experimentally. A survey of these reports and of the reports of contemporary and subsequent work on shock indicates that most of them dealt with a definable condition. The physiologic features compiled from these reports may be formulated into a useful definition:

Shock is a circulatory deficiency, not cardiac or vasomotor in origin, characterized by a decreased volume of blood and cardiac output and by hemoconcentration. The latter feature appears early, is easily determined and is seen in cases of moderate severity before variations in the blood pressure occur. For these reasons it is useful as an indicator. The following features are regularly associated when the syndrome is fully developed: lowered blood pressure and lowered basal metabolism, diminished renal excretion and an increased cardiac rate. In advanced stages the coagulability and the oxygen of the blood are decreased, the chlorides are below normal, and the nonprotein nitrogen is increased. Each of these associated features may occur in conditions other than

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1. Reports of the Special Investigation Committee on Surgical Shock and Allied Conditions, Medical Research Council, Special Report Series, nos. 25 and 26, London, His Majesty's Stationery Office, 1919.

shock, but their combination with the circulatory deficiency described seems to constitute a definite entity. I know of no other condition in which this combination occurs.

Another group of conditions are frequent but not constantly associated features: A decreased alkali reserve, as indicated by the carbon dioxide-combining power of the plasma, has been found in shock following surgical intervention, trauma, burns, anaphylaxis and other conditions. However, following intestinal obstruction there is agreement that the alkali reserve is increased. Variations in the temperature and in the respiratory rate are frequent but not consistently present. In shock following trauma, surgical intervention or certain abdominal emergencies the temperature is subnormal, but following burns it is increased. A similar increase may occur in shock associated with other conditions, but the increase may be due to the associated condition. In shock experimentally produced by various means our records did not show consistent variations. In many cases the temperature declined; in some it rose, and in some it showed little change. Clinical observations in shock following trauma, surgical intervention, burns and some other conditions in man indicate that the respiratory rate is increased. The same was true in some of our experiments on dogs. In others the respirations were slow and deep. Vomiting and diarrhea are usually present in severe shock. The vomitus, feces and urine often contain blood and albumin. In shock of lesser degree these features may be absent. Leukocytosis is frequent and is particularly marked following burns. In shock produced by injections of histamine there is transient leukopenia, followed by leukocytosis.[2] Whether leukocytosis always accompanies shock has not been shown.

For purposes of discussion and for comparison with other conditions it seems necessary to restate some observations previously reported.[3] Since distinctive changes are observed at necropsy in cases of shock, a summary of these will be given: The superficial veins are collapsed and bloodless. The blood in the heart and large vessels and in the parenchyma of organs is dark and thick and has failed to clot. Frequently it has a deep purplish cast. The serous surfaces are diffusely congested and appear cyanotic. In severe cases they contain ecchymoses, and the cavities contain blood-tinged fluid. The bowels are atonic, relaxed and distended. The vessels along the mesenteric attachment are engorged and prominent. The mucosae are congested, edematous and frequently contain ecchymoses. Their appearance resembles purple velvet. Occasionally there is bloody fluid within the lumen of the bowel.

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2. Moon, Virgil H., and Lieber, Marshall M.: Arch. Path. **20**:209-215, 1935.

3. Moon, Virgil H., and Kennedy, Patrick J.: Arch. Path. **14**:360-371, 1932; J. Lab. & Clin. Med. **19**:295-301, 1933. Moon, Virgil H., and Crawford, B. L.: Arch. Path. **15**:509-515, 1933. Moon, Virgil H., and Morgan, David R.: Arch. Surg. **32**:776-788, 1936. Moon, Virgil H.: Ann. Int. Med. **8**:1633-1648, 1935.

The fluid in the stomach may contain visible blood or brown flocculi. These contain hemoglobin, and the fluid contains serum albumin. The lungs are intensely congested either diffusely or in areas, producing a mottled appearance. Microscopically, the capillaries are distended and the venules are dilated and packed with corpuscles. Hemorrhages from the capillaries are numerous. Marked edema is present,[4] particularly if death has been delayed somewhat. The edema fluid has a high protein content, and its specific gravity approximates that of the blood plasma. The liver and kidneys are deeply congested. Blood oozes and drips from the parenchyma when this is sectioned. The cells regularly show parenchymatous degeneration. Patches of hemorrhage from capillaries occur in the medulla, and numerous red cells are observed within the tubules in such areas. This is an obvious source for the hematuria that is seen clinically.

The spleen usually contains less blood than it does normally. In dogs it is firm, contracted and dry. It serves physiologically as a reservoir. When there is need for blood, as in shock or in hemorrhage (fig. 2 *A* and *B*), the spleen discharges its surplus into the circulation. In shock the human spleen is flabby and the capsule wrinkled, but it is not so contracted and bloodless as in dogs. Apparently the human spleen is not so well supplied with musculature as that of dogs; hence it contracts less effectively.

The heart has a purplish, cyanotic color, and there is engorgement of the venules and capillaries in the myocardium. If death has been delayed somewhat the myocardial fibers show evidence of degeneration. Anoxemia is a regular feature in the late stages of shock. The parenchymatous degeneration seen in the myocardium, liver and kidneys may be due to anoxemia. When marked toxemia, as in acute infections, toxic jaundice, metabolic intoxications or drug poisoning has preceded the syndrome of shock, the toxemia may cause parenchymatous degeneration. However, when acute fatal shock has been produced in dogs by introducing muscle substance into the peritoneal cavity the myocardium shows only congestive changes, while the liver and kidneys show parenchymatous degeneration as well.

The adrenal glands participate in the capillary congestion seen elsewhere. Frequently there is vacuolation of the cortical cells, which was described by Zwemer[5] and others as resulting from a physiologic demand for cortical hormone, but the cells show no other changes. Recently some authors have attributed shock in general to adrenal insufficiency because the syndrome of shock with its characteristic associated features develops following extirpation of the adrenal glands

4. Moon, Virgil H., and Morgan, David R.: Arch. Path. **21**:565, 1936.
5. Zwemer, R. L.: Am. J. Path. **12**:107, 1936.

(Swingle and his collaborators [6]). However, in such experiments the shock is delayed and is not manifest until several days later. Hence if severe trauma, intoxications or burns by some hypothetic mechanism should completely obliterate all adrenal function the development of shock should not occur until several days later. In one of our experiments the implantation of muscle substance in the peritoneal cavity produced shock fatal in ten hours. Death from shock within twenty-four hours following injury is not infrequent. Obviously such results cannot be attributed correctly to adrenal insufficiency.

An alternate hypothesis would explain shock consequent to adrenalectomy as follows: One function of the hormone of the adrenal cortex may be to maintain the tonus of the capillaries and venules. Atony of these is regularly present in shock, and is the major factor in its development. These vessels become atonic and relaxed after a time when deprived of cortical hormone. Then a disparity between the volume of the blood and the capacity of the blood vessels develops because of the inability of the vascular system to contract with its normal tonus on the contained blood. Tonus and circulatory efficiency are restored by supplying the animal with extract of adrenal cortex. Loss of tonus and circulatory inefficiency reappear when this extract is withheld. Animals maintained on a minimal amount of the hormone of the adrenal cortex become abnormally susceptible to various agents and conditions which produce shock (Swingle and his collaborators). Apparently the minute vessels are more easily rendered atonic when supplied insufficiently with the hormone.

The function of other glands of internal secretion as well as the adrenals has been studied physiologically by noting the disturbances which follow their extirpation. If those disturbances disappear when an extract of the gland is supplied artificially, a function of that gland is thereby demonstrated. Similar logic might be applied to the results of experimentation on the adrenal cortex. The hypothesis that one of its functions is to maintain the physiologic tonus of the capillaries and venules deserves consideration.

The investigations of the committee appointed by the Medical Research Council [1] of wound shock during and following the World War established important facts, several of which are incorporated in the proposed definition. It was shown that the circulatory failure is not due to cardiac or vasomotor inefficiency. The heart in shock is able to maintain the blood pressure at or above normal levels if supplied with fluid. The vasomotor mechanism is active, and the peripheral

6. Swingle, W. W.; Pfiffner, J. J.; Vars, H. M.; Bott, P. A., and Parkins, W. M.: Science **77**:58-64, 1933. Parkins, W. M.; Taylor, A. R., and Swingle, W. W.: Am. J. Physiol. **112**:581-590, 1935. Swingle, W. W.; Pfiffner, J. J.; Vars, H. M., and Parkins, W. M.: ibid. **107**:274, 1934. Swingle, W. W., and Parkins, W. M.: ibid. **111**:426-439, 1935.

arteries are not atonic but are maximally contracted. It was shown that decreased volume of blood and hemoconcentration regularly accompany shock. The reports indicated that wound shock results from the absorption of injurious substances from traumatized tissue and that these substances affect the capillaries and venules in a fashion resembling the action of histamine. This interpretation was corroborated by the subsequent work of Cannon,[7] Krogh,[8] Lewis [9] and others and has been widely accepted. A recent review of the literature on the structure and function of the capillaries (Landis [10]) led to the generalization that any agent or condition injurious to capillaries renders their walls abnormally permeable to the plasma fluids. That generalization is exemplified by the experimental production of edema [4] by various agents injurious to capillaries. It is further corroborated by the occurrence of shock in diverse conditions associated with toxemia.

Our observations at necropsy in cases of shock in man and in animals support in every particular the interpretations just stated. The circulatory changes detailed earlier in this discussion were present following trauma; extensive surgical procedures; superficial burns; metabolic intoxications such as eclampsia, toxic jaundice and others; abdominal emergencies such as volvulus, mesenteric thrombosis, hemorrhagic pancreatitis and others; poisoning with various drugs, and infections of unusual severity. In each instance failing circulation and hemoconcentration preceded death. The same congestive and edematous features were produced experimentally in dogs by introducing muscle substance into the peritoneal cavity, by injections of watery extracts of muscle and of other tissues, by injections of bile and its salts, by burns, by intestinal obstruction, by deep narcosis with barbital and by injections of bacterial substances and of histamine. In each of these instances marked hemoconcentration preceded the circulatory failure.

From these considerations it appears that shock is a definite entity though it may originate from various sources, that it is accompanied by distinctive changes demonstrable both ante mortem and post mortem and that these changes are directly related to its mechanism of origin. Shock is simulated clinically by hemorrhage, syncope and rapidly developing cardiac failure. It presents pathologic features resembling those of passive congestion. Attention is invited to some particulars useful in the differentiation of these.

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7. Cannon, W. B.: Traumatic Shock, New York, D. Appleton and Company, 1923.

8. Krogh, A.: Anatomy and Physiology of Capillaries, ed. 2, New Haven, Conn., Yale University Press, 1929.

9. Lewis, T.: Blood Vessels of the Human Skin and Their Responses, London, Shaw & Sons, Ltd., 1927.

10. Landis, E. M.: Physiol. Rev. **14**:404-481, 1934.

SHOCK VERSUS HEMORRHAGE

Shock has been confused with exsanguination because of similarity in some of the clinical manifestations. In each condition the pulse and the respirations are rapid, the temperature is low and the blood pressure is diminished, but the conditions differ in important particulars:

Following hemorrhage the concentration of the blood at first is unchanged. Soon the concentration is lowered, as fluid is absorbed from the tissues into the blood. When fluid is supplied therapeutically it is retained in the circulation. No retention of nitrogen wastes occurs; hematuria does not occur, and the coagulability of the blood is not affected characteristically. No tendency to diarrhea or vomiting is present. At necropsy the tissues are pale, dry and anemic (fig. 1 *B*).

In shock hemoconcentration is present before circulatory inefficiency is manifested. Fluid is not absorbed from the tissues, and if injected into the circulation it tends to pass out rapidly, thereby accelerating the development of edema. The changes in the composition of the blood are as have been stated; the vomitus and excreta contain hemoglobin and albumin. At necropsy the viscera are congested and edematous and contain hemorrhages from the capillaries, as described in a preceding paragraph (fig. 1 *A*).

Frequently shock follows an injury in which blood has been lost, and hemorrhage may be a grave complicating factor. The circulatory phenomena of shock result from a disparity between the volume of the blood and the capacity of the blood vessels. Any loss of fluid such as that caused by hemorrhage following trauma or local loss of fluid as in burns will contribute to this disparity, and its importance will be proportionate to the amount of blood and/or fluid lost.

PRIMARY SHOCK

The term "primary shock" is sometimes applied to a condition characterized by a rapid, feeble pulse, shallow respirations, a low blood pressure and temperature and mental apathy or unconsciousness, occurring promptly following fright, grief, pain or minor physical injuries. There is marked weakness, sometimes accompanied by nausea and vomiting. Primary shock is essentially similar to syncope, and it has not been shown that these conditions are accompanied by a decreased volume of blood or by hemoconcentration. Neither have the visceral congestive features been shown which are characteristic of shock. Increased permeability of capillary walls seems to be an essential factor in the mechanism by which shock develops. Primary shock and syncope appear to be transient vasomotor phenomena in which no qualitative changes occur in the capillary walls or in the blood. These conditions

are not serious as compared with those grave conditions in which the minute vessels are both dilated and abnormally permeable. The clinical differentiation of primary shock should not be difficult, and the presence or absence of hemoconcentration should be diagnostic. Circulatory disturbances of vasomotor origin do not fall within the proposed definition for shock.

SHOCK VERSUS PASSIVE CONGESTION

It is a unique paradox that shock simulates exsanguination clinically, while its pathologic features resemble those of passive congestion. The similarity to passive congestion accounts for the failure of pathologists

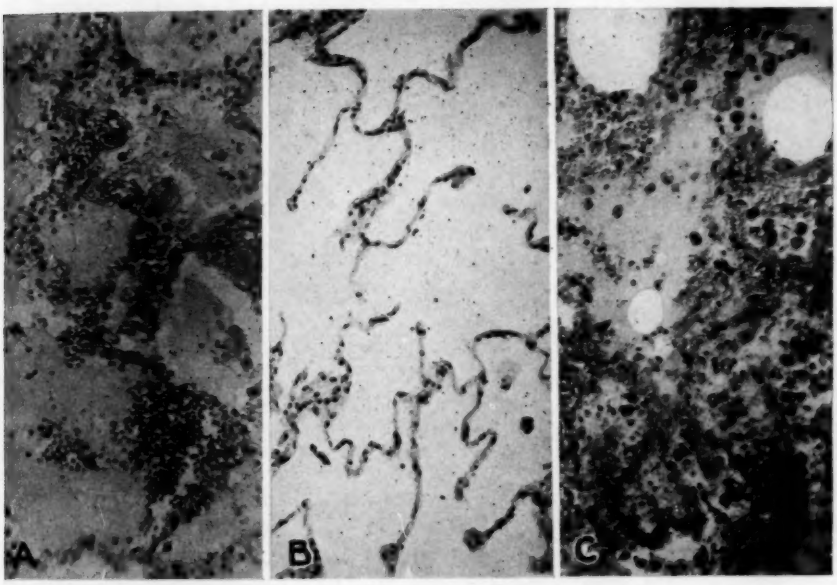

Fig. 1.—Photomicrographs of lungs: *A*, section made following death from shock, showing marked congestion, edema and capillary hemorrhage but no hemolysis or hematogenous pigment. *B*, section made following death from hemorrhage, showing no congestion or edema. *C*, section made following chronic passive congestion, showing features similar to those in *A* but with marked hematogenous pigmentation in addition.

to recognize the visceral changes of shock. A review of records of necropsies in cases of shock shows that the circulatory changes were adequately described, but invariably they were interpreted as passive congestion. Such interpretation is in accord with the principles of pathology as taught and practiced. I conformed to that practice until recently. Notwithstanding this similarity to passive congestion, the congestive, edematous and hemorrhagic phenomena of shock did not escape

the attention of those who recorded the pathologic changes due to burns. The descriptions by Long [11] in 1840 and by Bardeen [12] in 1896 of visceral changes following extensive superficial burns will apply without alteration to the changes accompanying shock from other causes.

Local passive congestion due to local venous obstruction need not be considered with reference to shock. Systemic passive congestion may have origin in the heart or in the lungs. Rapidly developing cardiac insufficiency, such as that resulting from coronary occlusion or from sudden incompetence of a valve, may present clinical features resembling those of shock. As these conditions are cardiac in origin they do not conform to the proposed definition. Usually the clinical signs are sufficient for the differentiation. The observations at necropsy in such a case would indicate clearly the cardiac origin of the circulatory failure.

The most common cause of passive congestion of longer duration is incompetence and/or stenosis of the mitral or aortic valve.. Moderate hemoconcentration may be present but the other clinical features are not those of shock. The observations at necropsy are distinctive. The valvular lesions are obvious, and the pulmonary congestion is chronic. It is usually accompanied by induration and regularly by hematogenous pigmentation. "Heart failure cells" (fig. 1 *C*) and hematogenous pigmentation in the pulmonary tissues do not occur incident to shock.

Circulatory failure may result from myocardial degeneration. This affects the right side of the heart characteristically. Dilatation of the left ventricle independent of aortic insufficiency is extremely rare. Dilatation of the right side of the heart results in *systemic*, not *pulmonary*, congestion. Cardiac dilatation is not a feature in shock. Roentgenologic examinations have shown (Eppinger [13]) that in shock the heart is smaller than normal. This is a logical result of the decreased volume of blood returned to it. The diminished volume output per minute is not due to cardiac dilatation or to insufficient contractile power. It has been shown repeatedly that in shock the heart is able to maintain the blood pressure and volume output at normal levels, if supplied adequately with fluid.

The pulmonary causes which may give rise to circulatory obstruction and thereby to systemic passive congestion are emphysema and fibrosis. In emphysema the necropsy shows voluminous, light, feathery, distended lungs, the characteristics of which may be distinguished readily from the heavy, congested, edematous lungs seen in shock. Marked diffuse fibrosis resulting from tuberculosis or some other chronic inflam-

11. Long, J.: London M. Gaz. **1**:743, 1840.

12. Bardeen, C. R.: J. Exper. Med. **2**:501-514, 1897.

13. Eppinger, H.: Wien. klin. Wchnschr. **47**:10-47, 1934.

matory process should be differentiated by the presence of that condition and by the absence of the usual circulatory features of shock in the pulmonary structures. Pulmonary circulatory obstruction due to diffuse fibrosis should not produce congestion and edema in the lung itself.

Passive congestion, regardless of its origin, produces engorgement of the spleen, while in shock the spleen is relatively bloodless (fig. 2). This is a distinctive feature of importance. Another feature is that although chronic cardiac or pulmonary lesions may result in circulatory inefficiency with hemoconcentration, they are associated with an increased

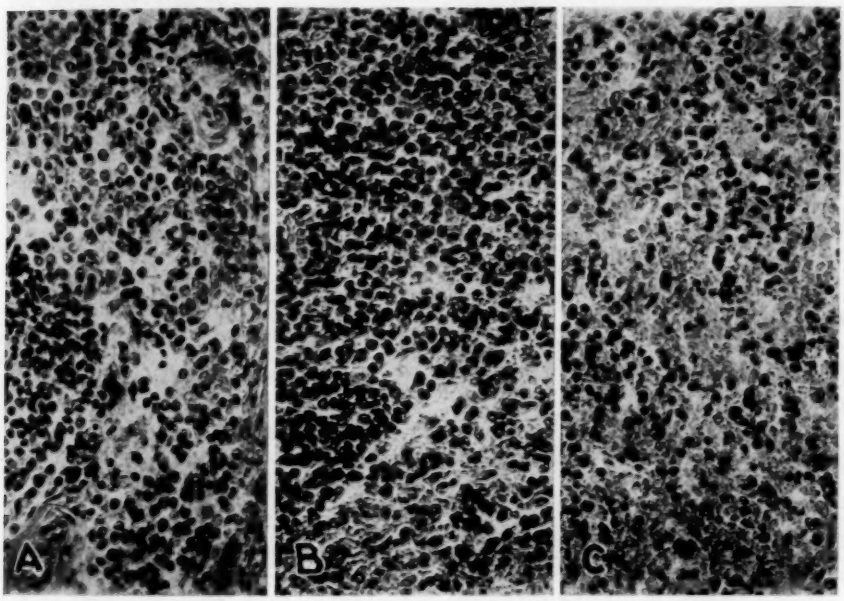

Fig. 2.—Photomicrographs of spleen. *A*, section made following death from shock. *B*, section made following death from hemorrhage. *C*, section made following death from passive congestion. It is seen that following shock and following hemorrhage the splenic pulp contains less blood than normal, while in passive congestion the spleen is engorged and contains hematogenous pigment.

total volume of blood—plethora. This feature is recognized by clinicians, and venesection is performed occasionally for its relief. In venous congestion the dilatation and engorgement affect the peripheral veins as well as those in the visceral areas. These are prominent and engorged before and after death. Postmortem lividity is marked. During shock the skin is pale, and it is difficult to obtain blood by venepuncture because of the collapsed condition of the superficial veins. The pallor of the

skin and the collapsed condition of the superficial veins persist after death except in the dependent portions of the body.

The postmortem observations following asphyxia are indistinguishable from those of shock. There is the same diffuse cyanotic congestion of mucous and serous membranes, accompanied by ecchymoses. The lungs, liver and kidneys are likewise engorged. There may be edema of the lungs and serous effusions. These changes may be interpreted as due to the same mechanism as the syndrome of shock. Asphyxia from whatever cause is accompanied by anoxemia, which of itself will cause dilatation and increased permeability of capillaries and venules (Krogh, Landis). Apparently asphyxia produces death by a mechanism similar to that of shock.

Observations drawn from a diversified postmortem experience lead to the conclusion that shock as defined is present as a terminal factor in the majority of deaths from all causes. The degree of this varies between wide limits. It is the dominant factor following extensive trauma to fleshy tissue and bone, extensive superficial burns, poisoning with certain drugs and severe toxemias. It is a minor terminal factor in various conditions of disease. The pulmonary congestion, edema and failing circulation which indicate to the physician that death is imminent are instances of the operation of the shock mechanism. These are due to vascular atony rather than to cardiac failure, which is the usual interpretation. Minor degrees of pulmonary congestion and edema are observed post mortem in such cases, while major degrees of these plus effusions and ecchymoses indicate that shock was the chief mechanism of death. Between these extremes the shock mechanism is operative in varying degrees of importance.

The term "congestive heart failure" used in the diagnosis of the cause of death comes near expressing the correct interpretation when shock is the major factor. The objection to this term is that in most instances the cause of death is not heart failure. With slight modification and qualification the term would serve adequately. "Congestive circulatory failure" due to whatever agent is concerned (trauma, burns, intestinal obstruction, toxemia) would express the interpretation clearly.

The congestion seen in the viscera should not be designated "passive congestion," since no hindrance to the return flow of blood is present. Neither should it be called "active congestion," since it does not result from an increased arterial blood flow. This dilemma may be escaped by using the term "acute venous congestion" in anatomic diagnoses to indicate that the changes seen are interpreted as due to the shock mechanism. The anatomic diagnosis at necropsy following death from burns in a typical case illustrates the use of these terms: "Congestive circulatory failure due to burns (cause of death); extensive second and third degree burns involving about two thirds of the total surface of the

skin; marked acute venous congestion of the lungs, gastro-intestinal mucosae, liver, kidneys and meninges; moderate anemia of the spleen; petechial hemorrhages in the pericardium, visceral pleura, and gastric mucosa and in the medullary substance of the cerebrum; acute parenchymatous degeneration of the liver, kidneys and myocardium; marked pulmonary edema; varying degrees of cutaneous edema."

SUMMARY

Shock is defined as a circulatory deficiency which is not cardiac or vasomotor in origin and which is characterized by a decreased volume of blood, by a decreased cardiac output and by hemoconcentration. Other physiologic disturbances regularly accompany it, but since their occurrence is not limited to shock they are not included in the definition.

It has been shown that deficiency of adrenal cortex produces shock. But the factor of time invalidates the supposition that such deficiency is the cause of shock occurring clinically or produced experimentally.

Shock may be differentiated clinically from hemorrhage and from syncope by observations on the concentration of the blood. The post-mortem observations will distinguish between hemorrhage, passive congestion due to pulmonary or cardiac disease and shock. These observations are etiologically related to the respective mechanisms involved.

In the absence of cardiac disease, the diagnosis of *congestive circulatory failure* as a cause for death is more accurate than that of *congestive heart failure*. The visceral engorgement characteristic of shock is not designated accurately either as active congestion or as passive congestion. It seems necessary to formulate a suitable term. "Acute venous congestion" is suggested as appropriate.

DIVERTICULUM OF THE GALLBLADDER

A REPORT OF THREE CASES

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Anomalies of the human gallbladder are infrequent, and diverticula are rare. The three cases reported here represent about 15 per cent of the total number recorded in the literature.

REPORT OF CASES

CASE 1.—A white woman aged 49 years was admitted to the Willard Hospital because of diffuse pinprick-like pain in the region of the shoulder blades of three weeks' duration. She had lost 32 pounds (14.5 Kg.) in the preceding six months. On admission she weighed 162 pounds (73.5 Kg.). Her previous illnesses were pneumonia and tonsillitis; after the latter illness she had had her tonsils removed. She had had three children and eight miscarriages.

Roentgenograms made twelve and sixteen hours after ingestion of an opaque test material showed a shadow of the gallbladder of satisfactory density. A line of negative density crossed it somewhat obliquely and seemed compatible with a band of adhesions. There was no evidence of nonopaque biliary calculi. Two hours after ingestion of a fatty meal the gallbladder had failed to empty completely, and the oblique line of negative density persisted. A diagnosis was made of chronic cholecystitis, paracolic adhesions and slow emptying of the gallbladder. At operation there were abundant adhesions about the gallbladder and its fossa. After cholecystectomy the patient made an uneventful recovery.

Pathologic examination disclosed the following: The gallbladder was 6 cm. long and 3 cm. across at the fundus. Its serosa was smooth and yellow from fat beneath it in about one half of its extent. In places the fat was as much as 4 mm. thick, while the wall elsewhere was only 1 or 1.5 mm. thick. The lining was normally rugate and without noteworthy gross change except for sparse stippling with golden yellow lipoid material. The cystic duct opened below the true neck in an ampulla in the body of the gallbladder, 1.5 cm. distal to the cephalad end. The neck ended in a blunt hollow cone about 8 mm. long. In the front wall of the fundus, pointing toward the liver, was an opening 8 mm. in diameter. This connected with a pouch, 2.7 cm. in diameter, lined with cholecystic epithelium and speckled with lipoid deposits (fig. 1). There was a ridge of mucosa 8 mm. high extending from the tip of the fundus to the most distal edge of the opening.

Histologically, the mucosa of the gallbladder was indistinct because of autolytic changes. Small pockets filled with desquamated epithelium were discernible in several places. The muscle and other layers were not inflamed. A portion of the diverticulum contained larger irregular cavities filled with desquamated epithelium. There was muscle in well defined layers and scattered wisps and evidence of

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From the Norman Bridge Pathological Laboratory, Rush Medical College of the University of Chicago.

chronic inflammation in the spongy serosa at the edge of the fossa. There were only occasional fat-containing specks in the tips of the plicae in both the gallbladder and its diverticulum in sections stained with sudan III and osmic acid.

CASE 2.—A white woman 52 years old, who had borne five children, entered the Willard Hospital with the following complaints: nervousness for three months; headache, difficulty in swallowing and abdominal pain for two months; diarrhea for two weeks, and a loss of 35 pounds (16 Kg.) in six months. She was apprehensive and irritable and felt that she was going to have "a nervous breakdown." The diarrhea amounted to from six to eight watery stools a day; the abdominal pain was generalized. For the preceding two months solid foods had been difficult to swallow; frequently they were vomited. The past history was essentially unimportant. The basal metabolic rate was +25, but the patient cooperated

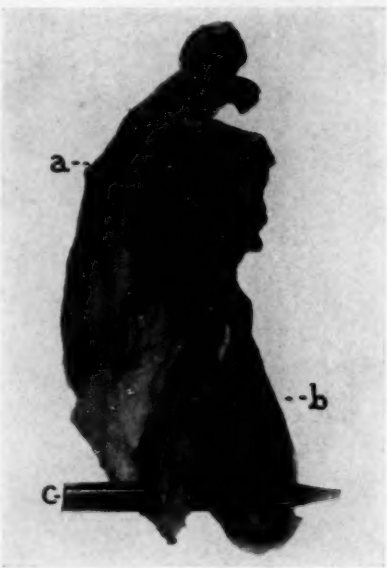

Fig. 1.—(*a*) Gallbladder. (*b*) Diverticulum. (*c*) Glass rod in an aperture between the two.

poorly. Roentgen studies of the upper portion of the gastro-intestinal tract ruled out stricture of the esophagus and gastric change. The patient was at times irrational and died of thyrotoxicosis twenty-six hours after her admission to the hospital.

Autopsy revealed many adenomas of the thyroid gland, some being calcified; cerebral edema, and arrested fibrous mitral and aortic endocarditis. The thyroid adenomas were of the fetal type, with small acini devoid of colloid. The gallbladder was 8.5 cm. long and 5 cm. across at the midportion. Its serosa was smooth and gray-pink except for a wrinkled, loose peritoneal fold, measuring 18 by 8 mm., at the fundus. The lining was dull brown in general, but the tips of the rugae in about a third of their extent were golden yellow from fat deposition (cholesterosis). There was an outpouching of the tip of the fundus in the direction of the hepatic attachment 2.4 cm. across and 1.4 high (fig. 2);

the vertical length of the pouch was 2.3 cm. For 1 cm. cephalad to the opening into the gallbladder, which was 1.3 cm. in diameter the diverticulum lay in contact with the gallbladder and at its upper edge was covered with the peritoneal fold mentioned. The diverticular lining was speckled with fat deposits in the same manner as the mucosa of the gallbladder.

Portions of the neck, body and fundus of the gallbladder and of the base of the diverticulum and septum were studied microscopically. In all sections there was a small lymphocytic increase in the submucosa, but such infiltration was exceedingly sparse in the muscularis. The fat was demonstrable in all sections,

Fig. 2.—(*a*) Gallbladder. (*b*) Diverticulum. (*c*) Its ostium.

being especially abundant in the tips of the plicae, but at times occupying a thin zone in the intervening mucosa. Some of the sections of the base of the septum were unusual. The mucosa consisted of two practically parallel layers separated by cavities containing mucus and epithelial débris. The chambers were bounded laterally by irregular columns of mucosa. The rugae projecting into the diverticular lumen were blunt, and some appeared to have fallen together to enclose a mucus-filled space.

CASE 3.—A woman aged 64 years entered the Willard Hospital with complaints of pain in the right upper quadrant of the abdomen, belching, constipation and loss of 8 pounds (3.5 Kg.) in four weeks. The attack occurred after she had

eaten a cold meal. Although the pain was relieved by rest in bed, it did not disappear and at times was felt in the right side of the back. The patient had noticed that the skin had been yellow for two weeks. As a child she had had to be careful of her diet, avoiding rich foods and carrots, for they initiated pain in the right side of the abdomen. At one time early in adult life she was given a hypodermic injection to alleviate an acute pain in the right side. On physical examination the edge of the liver was easily palpable 3 fingerbreadths below the costal margin, and a fist-sized mass occupied the region of the gallbladder. Seven and nineteen hours after the ingestion of opaque material roentgenoscopy revealed a faint round shadow in the region of the gallbladder. A similar shadow was seen four, seven and twenty-one hours after intravenous injection of opaque material. A diagnosis was made of pathologic enlargement of the gallbladder, without presence of calculi. Two hours after the fatty meal there was no change

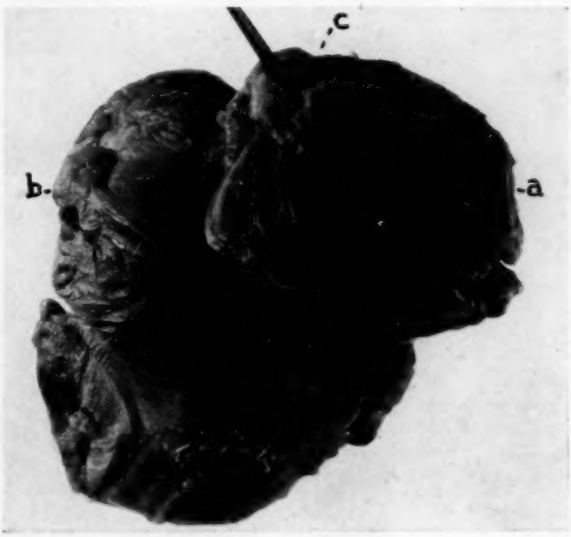

Fig. 3.—(*a*) Gallbladder. (*b*) Diverticulum. (*c*) Aperture containing a glass rod.

in the shadows, indicating a positive result of the Graham test. The icteric index was 22.

The gallbladder, removed after drainage by suction, was 12 cm. long and 6 cm. across at the fundus when collapsed. The material aspirated was straw colored, with flocculent yellow particles. After removal of the gallbladder and a constricting mass in the common bile duct the patient made a speedy recovery and was alive four months after operation.

The gallbladder contained two distinct cavities separated by a membrane lined with mucosa. The distal pouch measured 7 by 6 by 1 cm., the proximal pouch being about two-thirds as large. The communication between the two was through an oblique channel 15 mm. long, with an orifice 8 mm. in diameter (fig. 3). The mucosa of the channel was edematous. The proximal chamber was the gallbladder, and on the front side of its body was the aperture leading

into the diverticulum, which was larger than the gallbladder. The lining of both was speckled with lipoid deposits in the tips of the rugae.

Histologic examination revealed a small cell scirrhous carcinoma at the site of the obstruction. There was marked enlargement of the mucous glands of the wall of the duct bordering the new growth. There were edema and small

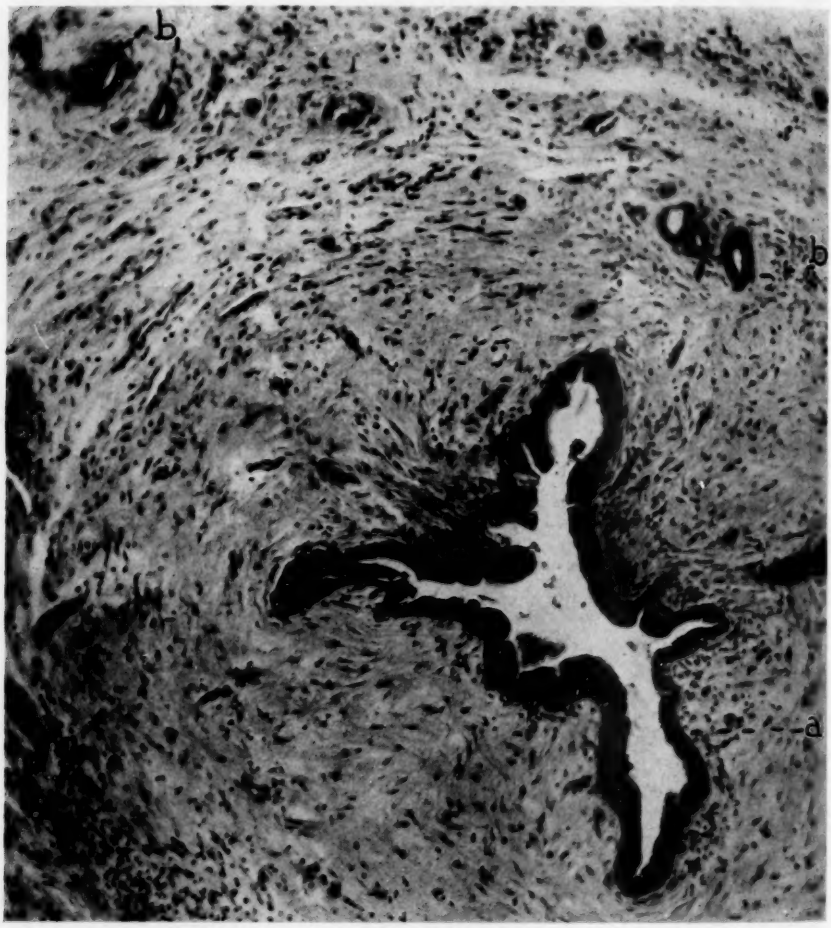

Fig. 4.—The wall of the diverticulum in case 3. At *a* is a large branched gland and at *b-b* are small Luschka ducts. The generalized lymphocytic infiltration is evident.

lymphocytic infiltration of both the diverticulum and the gallbladder and a moderate increase of connective tissue in the submucosa and muscularis. The zone about the channel connecting the two had a heavy, corrugated mucosa, with only shallow crypts between the crests of well preserved epithelium. Deep in the wall, occupying the muscularis and subserosa, were long ducts like bile passages (fig. 4). Some of these were as much as 0.2 mm. in diameter; most of them

were much smaller and possessed stubby branches. About some there were overgrowths of the loose fibrous connective tissue and small lymphocytic infiltration simulating the picture of pericholangeitis so common in the liver. There were smaller ducts of this nature, obviously Luschka's ducts, in the wall of the diverticulum where it lay in contact with the liver. The arteries in the wall of both the gallbladder and its appendage were markedly thickened by connective tissue in the media. The lumens were reduced to mere slits. Evidences of chronic inflammation were far greater in this diverticulum than in the preceding two.

COMMENT

The infrequency of anomalies of the human gallbladder has been repeatedly emphasized and is in marked contrast to their relative frequency in domestic animals. The huge series of mammalian gallbladders studied by Boyden [1] may well serve as a basis of comparison for the frequency with which anomalies occur in the various species. His observations were based on the examination of approximately 10,000 domestic animals (equal numbers of cats, calves, sheep and hogs) and reports of 19,000 cadavers and hospital patients. The incidence of duplication of the gallbladder was 1 in 8 cats, 1 in 28 calves, 1 in 85 sheep, 1 in 198 hogs and 1 in from 3,000 to 4,000 human beings. A comparative study of these anomalies in adults and embryos demonstrated trends of variation characteristic for each species. Boyden cast interesting sidelights on the subject and stated that aberrations of the gallbladder render an animal *tercfah* (unfit for food) among the Jews. The final authoritative sixteenth century codification of Talmudic law contained nine rules dealing with the absence or duplication of and concretions within the gallbladder.

Gay [2] proposed a gross classification for anomalies of the gallbladder, which include agenesis, variations in size and conformity, variations in position and reduplication of the organ. Dévé [3] followed much the same plan, as did Schachner,[4] Fowler [5] and Rolleston.[6] Schachner had perhaps the most elaborate classification of all. He placed in one group anomalies relating to the cavity of the gallbladder, as double gallbladder, bilobed gallbladder and diverticulum. Anomalies relating to the location of the gallbladder included intrahepatic, left-sided and floating gallbladders and transposition of the viscera. Individual anomalies were

1. Boyden, E. H.: Am. J. Anat. **38**:177-232, 1926.
2. Gay, R. J.: Tr. Chicago Path. Soc. **5**:108-113, 1902.
3. Dévé, F.: Bull. Soc. anat. **5**:261-270, 1903.
4. Schachner, A.: Ann. Surg. **64**:419, 1916.
5. Fowler, R. S.: M. Times **45**:230, 1917.
6. Rolleston, H.: Diseases of the Liver, Gallbladder and Bile Ducts, ed. 3, London, The Macmillan Company, 1929.

represented by hourglass gallbladders or absence of the organ. The most recent report in the literature was that of Gross,[7] who compiled data on 148 such abnormalities, including 9 diverticula.

The anatomic explanations for such diverticula fall into two groups: congenital and acquired. Those who believe in the congenital nature of these anomalies apply the results of studies on animal tissues directly to human malformations. The exponents of the acquired origin base their contentions on inflammation of the gallbladder. There is agreement, in general, in textbooks of histology[8] on the gross and microscopic structure of the gallbladder. Results of various investigations on the construction of the wall of the gallbladder and its blood and lymph supply also have been corroborated.[9] Disagreement is, however, most rife as to the mucosa of the gallbladder. Halpert[10] helped clarify the situation by his studies of the morphology of the gallbladder, wherein he indicated the differences between the Luschka ducts and the Rokitansky-Aschoff sinuses. A summary of some of Halpert's views is advisable.

> 1. "True Luschka ducts" are duct-like structures found occasionally in the periphery of the gall-bladder wall, occurring most frequently on the hepatic surface of the viscus and usually along the edges of the fossa vesicae felleae. They have a wall of their own with the histological structure of intrahepatic bile-ducts. The lumen is generally less than three-tenths of a millimeter in diameter and is usually lined with a somewhat lower columnar epithelium than that of the gall-bladder. . . .
>
> 2. "The Rokitansky-Aschoff sinuses" are deeper outpouchings or sinuses of the gall-bladder mucosa, which dip down into the muscularis or extend as finger-like processes through the muscular coat into the perimuscular layer.

Halpert noted the presence of Luschka's ducts in the gallbladders of 5 of 10 infants from 1 to 9 months of age; none in the bladders of 10 fetuses from 25 to 370 mm. long and in the gallbladders removed surgically from 1 of 120 patients from 16 to 75 years old. In 300 gallbladders surgically removed he and Mallory noted such ducts in nearly 10 per cent. These ducts have been demonstrated roentgenologically by Haberland.[11]

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7. Gross, R. E.: Arch. Surg. **32**:131-162, 1936.

8. Cowdry, E. V.: A Textbook of Histology: Functional Significance of Cells and Intercellular Substances, Philadelphia, Lea & Febiger, 1934. Jordan, H. E.: A Textbook of Histology, New York, D. Appleton-Century Company, Inc., 1934.

9. Sudler, M. T.: Bull. Johns Hopkins Hosp. **12**:126-129, 1901. Winkenwerder, W. L.: ibid. **41**:226-238, 1927.

10. Halpert, B.: Bull. Johns Hopkins Hosp. **40**:390-408, 1927; **41**:77-103, 1927; Anat. Rec. **32**:232, 1926.

11. Haberland, H.: Arch. f. klin. Chir. **89**:39, 1926.

The simplest explanation of epithelial overgrowths in the region of the gallbladder is offered by Budde.[12] He expressed accord with Hauser [13] and Boyden [1] concerning the early solid stage of the anlage of the gallbladder. He gave as his belief, moreover, that the entire ductus choledochus from the mouth of the pancreatic duct comprises the field of the primitive liver and that the glands regularly present in its wall are sprouts of the liver. The gallbladder is a differentiated bile duct the epithelium of which is the pacemaker in a mesenchymal field. Thus, there are inverting growths, the rugae, and everting growths, the Luschka ducts, and Rokitansky-Aschoff sinuses. Retrogression is marked in the hepatic sprouts near the duodenum, where the glands they form are unbranched and lie in the adventitia of the gallbladder. Arrest of growth is less extensive as the liver is approached; even in the region of the cystic duct such glands have anastomosing ducts and numerous end-pieces. Budde reported a diverticulum of the common bile duct which served as a rudimentary gallbladder when all vestiges of a cystic duct or bladder were absent.

A rich glandular provision was found by Shikinami [14] for the entire gallbladder of the hog, sheep and calf but in human beings he noted such glands only at the neck of the bladder. He stated that he was convinced that excessive growth of Luschka's ducts is responsible for adenomas and diverticula of the gallbladder. Such adenomatous inclusions were mentioned also by Hauser,[13] who quoted Luschka to the effect that these are due to inflammation. This premise is not accorded recognition in some of the standard discussions of cholecystitis.[15] Herxheimer [16] observed normal glands in circumscribed regions deep in the wall of the gallbladder and regarded this heteroplasia on a developmental basis. He also referred to R. Meyer, who reported epithelial rests in the gallbladder of a human embryo 8.7 mm. long. Hartmann [17] reviewed the work of many of the opponents in the controversy over the significance of Luschka's ducts and adenomas in the production of diverticula. He cited Aschoff and Bacmeister, who have stated the

12. Budde, M.: Arch. f. klin. Chir. **128**:586, 1924.

13. Hauser, R., in Henke, F., and Lubarsch, O.: Handbuch der speciellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1929, vol. 5, pt. 2.

14. Shikinami, J.: Anat. Hefte **36**:551-597, 1908.

15. Cheney, W. F.: Diseases of the Gall Bladder, in Piersol, G. M.; Bortz, E. L., and others: Cyclopedia of Medicine, Philadelphia, F. A. Davis Company, 1933, vol. 8, p. 283. Lauda, E.: Cholangitis, ibid., vol. 8, p. 311. Rolleston, H.: Diseases of the Gall Bladder and Bile Ducts, in Christian, H. A., and Mackenzie, J.: Oxford Medicine, London, Oxford University Press, 1932. Kaufmann, E.: Pathology, Philadelphia, P. Blakiston's Sons & Co., 1929, vol. 2, pp. 984-997.

16. Herxheimer, G., in Schwalbe, E.: Die Morphologie der Missbildungen des Menschen und der Tiere, ed. 10, Jena, Gustav Fischer, 1913, pt. 3, p. 249.

17. Hartmann, H.: Fortschr. a. d. Geb. d. Röntgenstrahlen **52**:30-36, 1935.

opinion that adenomatous inclusions of the wall of the gallbladder are congenital malformations in the manner of Meckel's diverticulum. They noted such adenomas containing cholecystic epithelium or mucous glands in 3 per cent of the gallbladders they studied. Lubarsch, in his series, noted adenomas in 5 per cent of the gallbladders studied. He usually saw them encircled by thickened muscle—the picture of an adenomyoma. He stated that he believed he was dealing with epithelial hypertrophy or heterotopic epithelial hyperplasia resulting from inflammation. Nikod of Mayenburg observed identical structures but ascribed a minor rôle to inflammation in their production. Diverse opinions respecting the origin and structure of glandular and duct elements in the wall of the gallbladder are applied in explaining the formation of cholecystic diverticula.

Frequent reference is made to the work of Weltz,[18] who studied 5 diverticula. His largest specimen was 15 mm. long and 8 mm. in diameter and projected for 3 mm. Its lumen was filled with yellow detritus and bile and was connected with the fundus. One cavity that he classed as a diverticulum possessed no connection with the lumen of the gallbladder; one pouch was visible only after an angular slit which guarded its mouth had been spread. Stones and marked inflammation were present in 3 of the pouches; hyperplasia of the lining was noted in 4, and adenoma, in 1. Weltz, although stating that he was intrigued by the possibility of cyst formation in the glands of the wall of the gallbladder as a result of inflammation, decided that in his heterogeneous collection the sacs were of congenital origin. The huge diverticulum reported by Toida[19] forms a marked contrast to the small evaginations enumerated. He found a sac 8 cm. in diameter connected to the gallbladder of a girl 5 years old, who had been operated on for supposed hydronephrosis. The stalk of the pouch was rich in Luschka's ducts, poor in blood vessels and devoid of inflammation. The wall of the pouch was fibrous, it contained no muscle, it was lined by cylindric epithelium and was filled with bile. Congenital weakness of the wall of the gallbladder was considered the cause of this diverticulum.

Diverticula of the biliary vesicle are the source of a variety of pathologic changes and surgical complications. Michalski[20] encountered a stone the size of a cherry in a sacculation of the gallbladder in a woman who had had indefinite "rheumatic" pains in the back. Abbott[21] noted a diverticulum one-half inch (1.5 cm.) deep at the neck of a gallbladder which was bound to the duodenum. Inflammation was present

18. Weltz, G. H.: Ueber Divertikel der Gallenblase, Kiel, L. Handorff, 1894.
19. Toida, R.: Arch. f. klin. Chir. **100**:1188, 1913.
20. Michalski, J.: Schweiz. med. Wchnschr. **1**:492, 1920.
21. Abbott, G. K.: Surg., Gynec. & Obst. **36**:466-467, 1923.

only in the diverticulum and had spared the bladder. In other instances marked chronic inflammation of the bladder accompanied similar changes in the offshoot. Pertl [22] reported a case in which the diverticulum had perforated the jejunum. A stone, three-fourths inch (2 cm.) in diameter blocked the cystic duct, apparently paving the way for the development of marked hydropic tension. A more serious pressure effect by a diverticulum was discovered by Schening and Schondube.[23] They were forced to resect a part of the stomach because of the intimate adherence between the entire gallbladder and the gastric antrum. They found a hydrops of the gallbladder due to impaction of a stone the size of a pea in the cystic duct. A diverticulum the size of a man's fist with a stalk the size of a pencil was connected with the fundus of the gallbladder. Coarse rugae in the wall of the sac surrounded recesses containing gallstones. The fundal partition separating the gallbladder from the pouch contained many cystically dilated Luschka ducts. The authors classed this as an acquired diverticulum which started in an adenoma of the fundus. They traced the course of events as follows: Cholecystitis led to the development of inflammatory adhesions between the gallbladder and the stomach. After occlusion of the cystic duct, hydrops led to increased pressure within the gallbladder. The distensibility of the gallbladder was reduced by scar, but a locus of lowered resistance existed in an adenoma at the fundus, and a pouch formed. The adenoma could no longer be demonstrated, although it may have originated in one of Luschka's ducts.

Persistent cystic ducts of the liver forming a direct connection between the liver and the gallbladder were reported by Eisendrath,[24] who observed a diverticulum at the neck of the gallbladder. Nadeau [25] dissected a diverticulum which perforated the front surface of the liver and was connected with the fundus of the gallbladder. There were no signs of marked inflammation in the sac, but the fundus of the gallbladder was adherent to the colon. The cystic duct was sharply kinked by adhesions to the duodenum. The site of this diverticulum can be ideally explained by adenomatous or cystic alterations in Luschka's ducts. Gross [7] suggested that remnants of these ducts, which ordinarily face the liver, may lie on the exposed surface of the gallbladder as a result of normal rotation of the gallbladder.

Pseudodiverticula of the gallbladder are of several varieties. The most common are the eventrating pouches associated with cholecystitis.

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22. Pertl, A. L.: Ann. Surg. **98**:318-320, 1933.
23. Schening, W., and Schondube, W.: Arch. f. klin. Chir. **137**:308-314, 1925.
24. Eisendrath, D. N.: J. A. M. A. **71**:864, 1918.
25. Nadeau, O. E.: Med. & Surg. **1**:73, 1917.

Ross [26] described such a pouch, 1 cm. in diameter, in the fundus of the gallbladder near the liver. The wall of the sac contained muscle and connective tissue but no epithelium. The tentative explanation for the condition was that an inflamed and ulcerated surface had overhanging mucosal borders. These surfaces adhered to the interior of the pouch thus formed and healed, enclosing a persistent tract into the gallbladder. The vesical epithelium failed to grow sufficiently to line the cavity. The inflammatory cul-de-sacs, at times extremely numerous and filled with calculi, belong also to this group. They are associated with injutting partitions, the result of epithelial stimulation.

A second group of pseudodiverticula are anatomically elusive but have been studied roentgenographically. These forms often are apical and visible as snared-off portions in the long axis of the gallbladder. Bársony,[27] who stated that he believed a muscle spasm or a constriction from adhesions might account for the picture as readily as the presence of a diverticulum, was unable to confirm his roentgenologic observations by dissection. Chiray and Loman [28] explained minutely the differential roentgen diagnosis of cholecystic diverticulum from a congenital transverse septum, which may produce slight retraction at its insertion into the wall of the gallbladder.

The presence of lipoid deposits in the mucosa of the gallbladders and diverticula noted in my cases was to be anticipated from the investigations of Mentzer.[29] He reported lipoid accumulations in the gallbladders of 53.9 per cent of women. The association of fatty changes in the body with pregnancy is generally conceded; the specimens were obtained from multigravid women. The cholesterosis noted is therefore considered merely incidental and not related to the formation of the diverticulum. But the similarity of the fat deposition in the lining of the gallbladder and its appendages seems highly significant in establishing a common change in these chambers.

The functional ability of the diverticulum was proved by roentgen visualization in cited cases 1 and 3. Enough dye was excreted in the bladder to permit the study and diagnosis of a pathologic condition. There was prompt relief from symptoms after cholecystectomy.

With the exception of the region of the connecting channel in case 3, the inflammatory alterations of the gallbladder were of about the same grade as those of the diverticulum. This condition speaks, therefore, for a congenital defect of long duration which eventually was

26. Ross, G.: Ann. Surg. **63**:627, 1916.

27. Bársony, T.: Fortschr. a. d. Geb. d. Röntgenstrahlen **36**:759, 1927.

28. Chiray, M., and Lomon, A.: Presse méd. **42**:1559-1561, 1934.

29. Mentzer, S. H.: Am. J. Path. **1**:383-388, 1925.

subjected to inflammation. The stalk zone in case 3 possessed potentialities for the formation of a cyst or adenoma in far greater abundance than did the partition zones in cases 1 and 2.

SUMMARY

Three cases of congenital diverticulum of the gallbladder are reported. Each diverticulum possessed the same degree of chronic inflammation as was noted in the gallbladder. Glands possessing potentialities for the formation of a cyst or adenoma were encountered in all the diverticula and in one common bile duct (case 3) in which a carcinoma existed.

FOCAL AND GENERAL TISSUE RESPONSES TO AN AVIRULENT TUBERCLE BACILLUS (BCG)

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CHICAGO

The histogenesis of the tubercle was surmised long before the tubercle bacillus was isolated (Bayle,[1] Laennec,[2] Villemin [3]). Since Koch's discovery the studies dealing with this reaction have been innumerable (Baumgarten,[4] Yersin,[5] Huebschmann,[6] Krause,[7] Maximow [8] and others).

The question of the sequence of the cellular responses has given rise to much discussion without universal agreement. Whether exudation with polymorphonuclear predominance (Huebschmann,[6] Yersin [5]) or proliferation of the tissue with histiocytic superiority (Baumgarten,[4] Krause [7]) has priority in the formation of the tubercle is still unsettled. In the studies reported the varying experimental conditions (the number of bacilli employed, the mode of inoculation and the organs examined) have made for the majority of the discrepancies.

In the effort to solve this basic problem another phase of the pathology of tuberculosis has been neglected, namely, the remote response of the tissues where tubercle bacilli are not demonstrable by microscopic examination or by tissue cultures. This response, as the following studies indicate, is mainly concerned with the reticulo-endothelial system. That such a reaction might occur is strongly suggested by the fact that no matter what the method of administration of the tubercle bacilli may be a generalized allergic response is manifested

From the University of Illinois College of Medicine.

From the Tice Laboratories, a detail of the Municipal Tuberculosis Sanitarium.

1. Bayle, G. L.: Recherches sur la phthisie pulmonaire, Paris, Gabon, 1810.

2. Laennec, R. T. H.: Traité de l'auscultation médiate et des maladies des poumons et du coeur, ed. 3, Paris, J. S. Chaudé, 1931.

3. Villemin, I. A.: Gaz. hebd. de méd. **2**:50, 1865.

4. Baumgarten, P.: Ztschr. f. klin. Med. **9**:93, 1884.

5. Yersin, M. A.: Ann. Inst. Pasteur **2**:245, 1888.

6. Huebschmann, P.: Pathologische Anatomie der Tuberkulose, Berlin, Julius Springer, 1928.

7. Krause, A. K.: Am. Rev. Tuberc. **15**:137, 1927.

8. Maximow, A. A.: J. Infect. Dis. **34**:549, 1924; Tr. Nat. A. Prev. Tuberc. **11**:342, 1925.

within a short period. The rôle of the reticulo-endothelial system in immunity was known to Metchnikoff,[9] Ehrlich[10] and Levaditi.[11] That it is an important factor in immunity is conceded generally (Aschoff,[12] Maximow,[8] Jaffé[13]).

The local tubercle formation is dependent to a large extent on the reticulo-endothelial system (Goldmann,[14] Kiyono, Evans,[15] Bowman, Winternitz, Lang,[16] Maximow[8]). Cunningham, Sabin, Sugiyama and Kindwall[17a] expressed the belief that the epithelioid cells of the tubercle are derived from the monocytes of the blood, a view also held by Vorwald.[18]

When virulent tubercle bacilli are injected into a susceptible animal locally the organisms spread rapidly, and generalized tuberculosis may result from the consequent bacillemia. Thus, Coulaud[19] and Nakada[20] claimed to have produced primary complexes of the lung when they injected the bacilli subcutaneously or gave the organisms by mouth.

The problem presented in this work is: What is the reaction in remote parts of the body, particularly in the susceptible tissue in the sense of Besredka, when an avirulent organism (BCG) which tends to remain localized is injected? Also, do tubercles in various organs develop similarly? An attempt is also made to correlate the humoral and the tissue responses.

METHOD

Guinea-pigs were inoculated intracardially with from 10 to 15 mg. of BCG and killed at intervals of ten minutes, one, three, five, eight, sixteen, twenty-four, thirty and forty-eight hours, three, five, seven, nine and fifteen days and one,

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10. Ehrlich, P., quoted by Aschoff.[12a]

11. Levaditi, quoted by Aschoff.[12a]

12. (*a*) Aschoff, L.: The Reticulo-Endothelial System, in Lectures on Pathology, New York, Paul B. Hoeber, Inc., 1924. (*b*) Aschoff, L., and Kiyono, K.: Folia haemat. **15**:383, 1913.

13. Jaffé, R. H.: Physiol. Rev. **11**:277, 1931.

14. Goldmann, E. E.: Beitr. z. klin. Chir. **64**:192, 1909.

15. Evans, H. M.; Bowman, F. B., and Winternitz, M. C.: J. Exper. Med. **19**: 283, 1914.

16. Lang, F. J.: J. Infect. Dis. **37**:430, 1925.

17. (*a*) Cunningham, R. S.; Sabin, F. R.; Sugiyama, S., and Kindwall, J. A.: Bull. Johns Hopkins Hosp. **37**:231, 1925. (*b*) Cunningham, R. S., and Tompkins, E. H.: Am. Rev. Tuberc. **17**:204, 1928.

18. Vorwald, A. J.: Am. Rev. Tuberc. **25**:74, 1932.

19. Coulaud, E.: Rev. de la tuberc. **1**:719, 1933.

20. Nakada, Y.: Beitr. z. Klin. d. Tuberk. **83**:377, 1933.

two, three and fourteen months. Histologic examinations of the lungs, lymph nodes, liver, spleen, kidneys and heart were made. Large sections were taken, usually through the entire organ. Several lobes of the lungs were sectioned, each section being an entire vertical section of the lobe. Concomitant whole blood counts and differential counts as well as tuberculin tests were made at frequent intervals.

Each animal was killed by a single blow on the occipital region. The tissues were fixed in Zenker's solution, and the lungs in situ received an injection of Zenker's solution before the chest was opened. The tissues were all embedded in paraffin, and sections were stained with hematoxylin and eosin and with carbol-fuchsin and methylene blue.

Control animals of varying ages (ten animals from 2 days to 1½ years old) were studied histologically in the same way.

The macroscopic studies will be discussed later. Summaries of the microscopic studies follow:

LUNG

Reaction of Tubercle Bacilli.—The original injected bacilli were long, slender and slightly granular. Within a few minutes after injection they appeared within the blood vessels singly or in small groups, but after a few hours they were found in large clumps. The size of these groups of bacilli increased up to about forty-eight hours. The numbers of organisms decreased after three days. At five days they were scarce. By the end of the fifteenth to twenty-fifth day they could not be found on microscopic examination. The bacilli had gradually changed their forms to become short, plump and heavily beaded with an ever-increasing number of acid-negative granules. Toward the end only granules were noted, which were both acid-fast and nonacid-fast. Cultures of a mixture of the organs were positive up to and including twenty-five days. Cultures of the blood were positive up to forty-eight hours.

Reaction of Host.—1. Tubercle formation in the presence of numerous bacilli: Almost immediately after injection of the bacilli an intracapillary leukocytosis was evident, and the bacilli were found within the vessels, both free and within granulocytes. Rarely were they contained within monocytes. The bacilli, free or engulfed, became lodged in the capillaries of the alveolar walls, where more granulocytes gathered and surrounded or phagocytosed the organisms. By the third hour swollen histiocytes (probably of septal cell origin) made their appearance in small numbers and were found to ingest leukocytes with or without bacilli. By the sixteenth hour the areas of bacillary localization had progressed to nodular swellings composed of a central portion of bacilli surrounded by a palisade-like arrangement of granulocytes. The latter seemed to send long processes into the bacillary mass, while their nuclei arranged themselves in the distal portions of the cells, forming a rosette-like structure. Swollen histiocytes were present to the extent of 15 per cent, usually toward the periphery of the nodule. Up to this point, the process may still have been intracapillary, depending on the localization. That is, if the latter was within the thin part of the alveolar septums, aneurysmal outpouchings occurred (fig. 2) which finally ruptured into the alveolar spaces (fig. 3). Hemorrhage did not occur because by this time the capillaries were sealed with fibrin. If the process was found at the junction of alveolar walls, the tubercle in many instances continued intramurally and did not rupture into the alveolar spaces.

At from twenty-four to seventy-two hours the numbers of granulocytes and histiocytes forming the tubercle were about equal (fig. 4), and phagocytosis of the former by the latter was frequent. At the periphery of the nodule the epithelioid cells predominated, and some of them contained tubercle bacilli, which formed the focus of purely epithelioid cell proliferations.

By the end of the first and the beginning of the second week multinuclear giant cells as well as Langerhans' giant cells made their appearance. The tubercle at this stage was usually composed of epithelioid cells and lymphocytes, but an occasional granulocyte was found, especially at the periphery of the nodule. In the presence of many organisms, polymorphonuclear leukocytes were found in greater numbers (fig. 5).

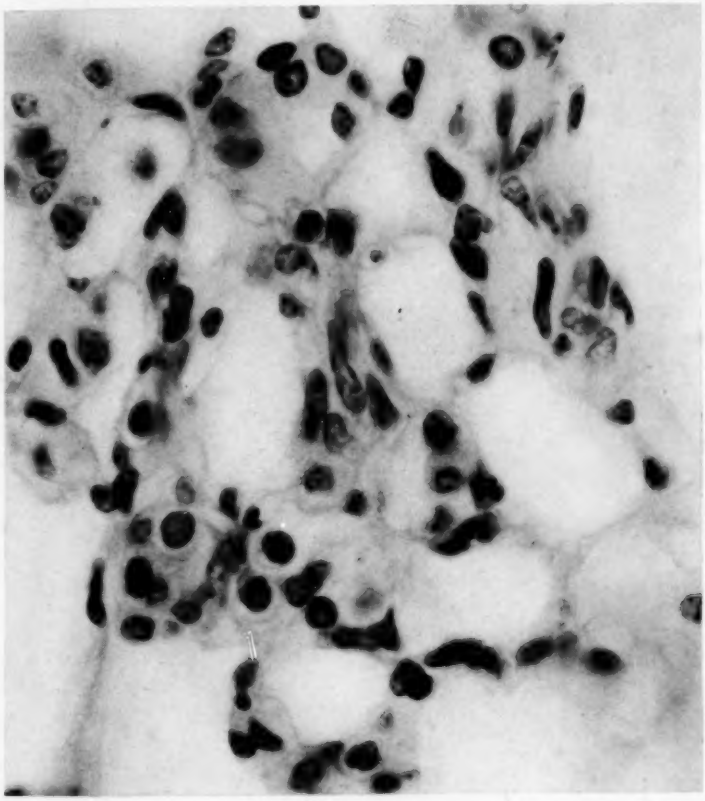

Fig. 1.—Lung one and one-fourth hours after intracardial injection of bacilli; × 500. The septal cells are swollen and have arranged themselves along the inner aspect of the alveolar walls. Note the dark-staining nuclei of the capillary endothelium in contradistinction. This section and those shown in the subsequent figures were stained with hematoxylin and eosin.

From the fifteenth to the twenty-fourth day bacilli as well as granulocytes were rare. The tubercles composed of giant cells, epithelioid cells and lymphocytes

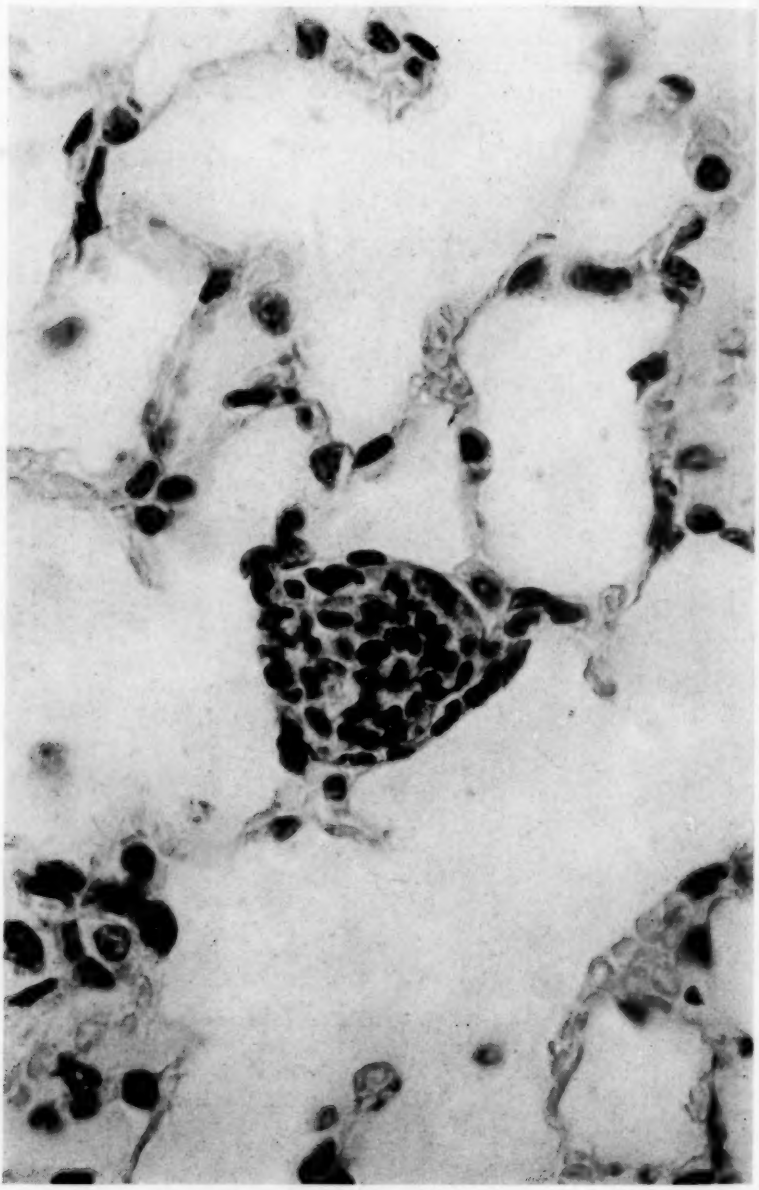

Fig. 2.—Lung three hours after injection of bacilli; × 500: An intracapillary and intramural focus of tubercle bacilli and polymorphonuclear leukocytes. This aneurysmal outpouching is from the midportion of the alveolar wall. The septal cells in the vicinity show rounding while the capillary endothelium is unaffected.

Fig. 3.—Lung sixteen hours after injection of bacilli; × 500: Rupture of an aneurysmal nodule such as that shown in figure 3 has occurred. There is no hemorrhage. Granulocytes and swollen histiocytes are present.

were smaller than before. The reduction in the size of these nodules gradually progressed as the number of lymphocytes increased proportionately (fig. 6), so that by the end of the first month, rarely the second month, one still found an occa-

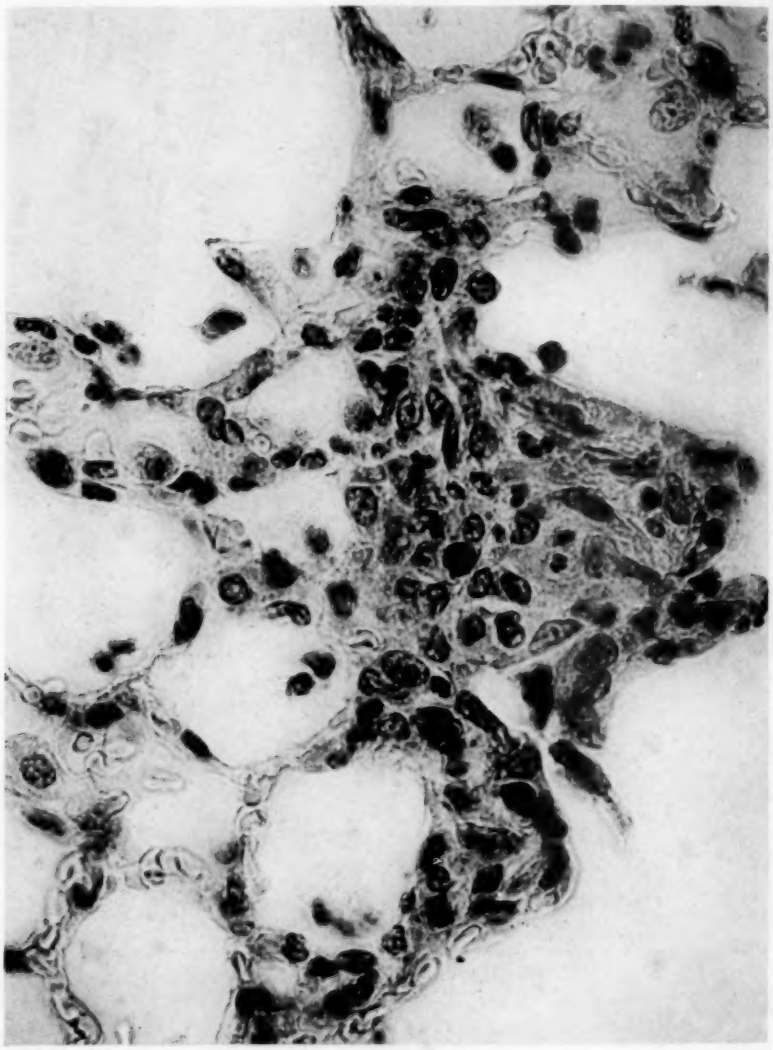

Fig. 4.—Lung forty-eight hours after injection of bacilli; × 500: A tubercle showing a central granulocyte and intracellular tubercle bacilli and a preponderance of epithelioid cells in the periphery. Swelling of septal cells remote from the tubercle can be seen.

sional giant cell containing acid-fast granules and surrounded by a few epithelioid cells and lymphocytes. These, too, had disappeared by the end of the third month. Fibrosis or caseation rarely occurred, and restitution was complete.

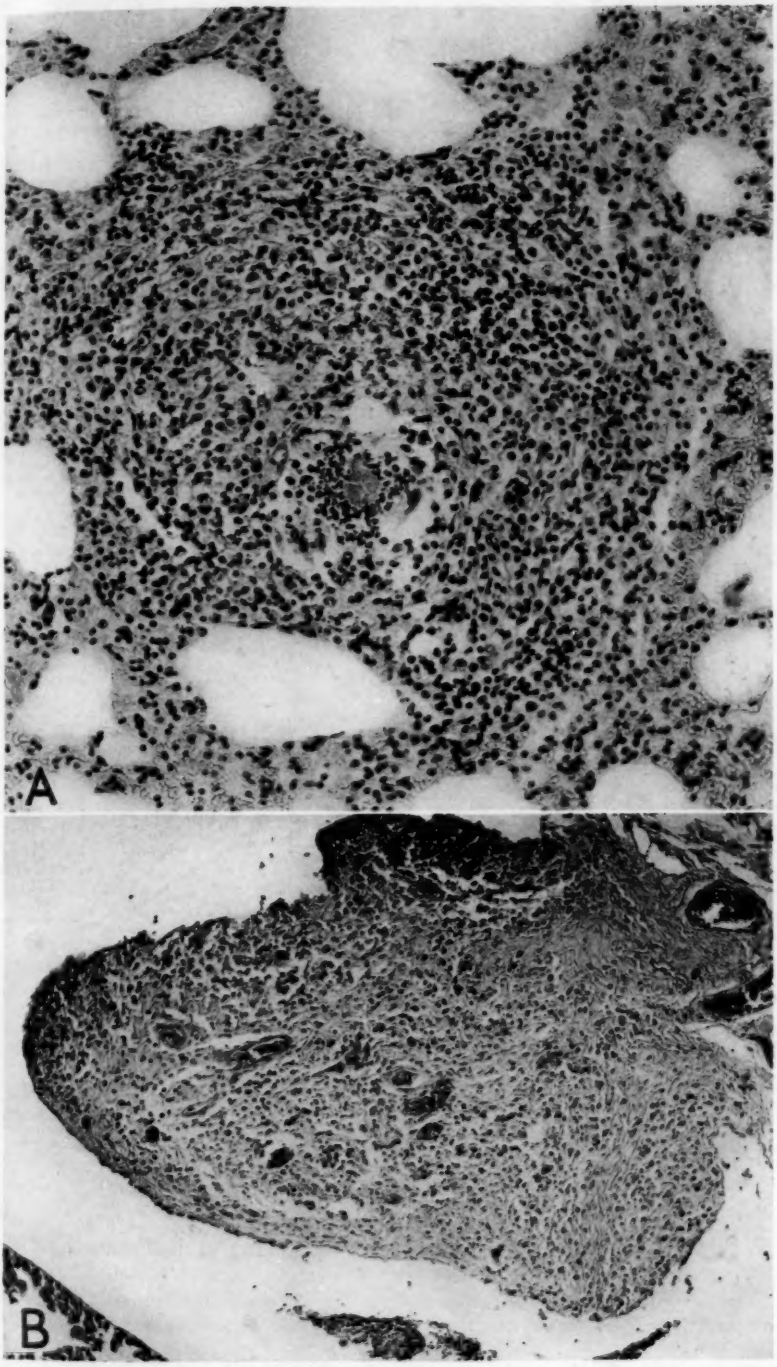

Fig. 5.—*A*, lung nine days after injection of bacilli; × 200: A large tubercle containing tubercle bacilli and degenerated pus cells centrally, surrounded by giant cells, epithelioid cells and finally lymphocytes. *B*, hilar lymph node at nine days; × 150: Langerhans' giant cells, epithelioid cells and lymphocytes replace the node. Both figures represent the same animal.

2. Tubercle formation in the presence of few bacilli: Single bacilli were at times found in the centers of lymph follicles of the lung, within granulocytes. The proliferation about the organisms was reticulum cell in character, from the germinal centers. Single bacilli were also encountered at the periphery of the tubercle engulfed by epithelioid cells after forty-eight hours. At times these cells were remote from the tubercle. Such histiocytic cells were surrounded mainly by proliferated histiocytes, epithelioid in character, although in many instances no reaction was noted. The regression of such nodules was similar to that already described with the infiltration of lymphocytes. Fibrosis was absent.

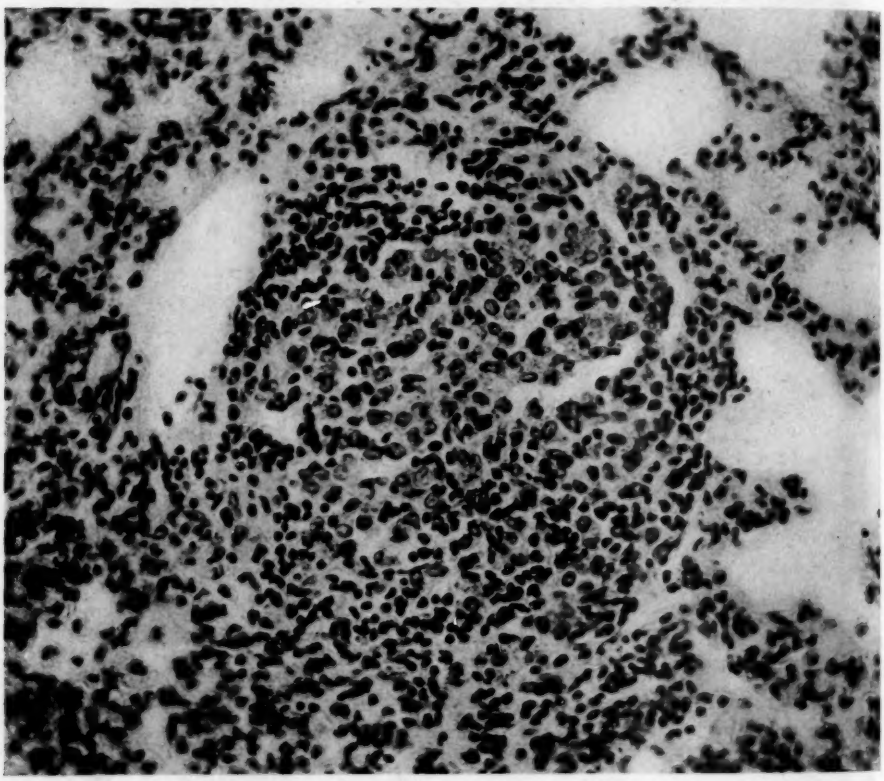

Fig. 6.—Lung fifteen days after injection of bacilli; × 250: A tubercle composed of epithelioid cells, surrounded and invaded by lymphocytes.

3. Reaction of reticulo-endothelial system in areas without demonstrable bacilli: Swelling of the septal cells began almost at once (fig. 1) after the injection of the bacilli. The swelling progressed slowly and was well pronounced by the third hour. The cells became swollen, protruding into the lumens of the alveoli as small nodules. Their cytoplasm became somewhat basophilic. A similar histiocytic swelling occurred about the smaller vessels. This process remained very active during the formation of the tubercle (from three to fifteen days) and was evident after the tubercle had subsided. At this time histiocytic cellular accumulations were noted in the form of small cushions that protruded into the lumens of the alveoli.

Such a process, but less extensive, was found in the lung of an animal killed three months after injection of the bacilli. However, this animal had shown Koch's phenomenon from old tuberculin, and the wound had not yet completely healed (fig. 7). At fourteen months no noticeable septal cell reaction could be found.

Throughout the period of observation the capillary endothelium could be clearly distinguished from the septal cells; the former did not enter into the reaction, the cells remaining spindle-shaped with dark-staining nuclear chromatin.

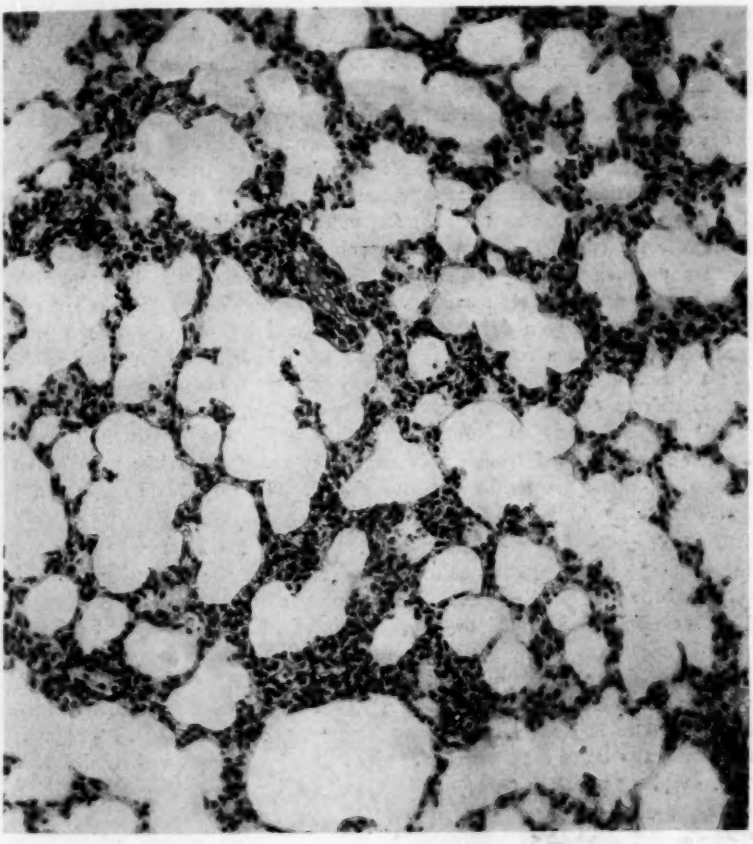

Fig. 7.—Lung three months after injection of bacilli; × 125: Moderate hyperemia and septal cell swelling following Koch's phenomenon due to old tuberculin.

In control animals the septal cells were pavement-like, and the chromatin of their nuclei stained deeply. At times one found areas of subpleural atelectasis, in which dense accumulations of cells occurred. These accumulations were well localized and should not be confused with the more diffuse reactions described.

LIVER

Reaction of Tubercle Bacilli.—There was no evidence of increase in the number of bacilli as in the lung. The organisms were never numerous and were

usually free in the capillaries or within granulocytes. After three hours the tubercle bacilli were noted within the Kupffer cells. They were of normal appearance. At five hours many granules were noted within the bacilli, and this process increased until forty-eight hours, when the granular forms were the rule. Although an occasional bacillus was noted in sections later than forty-eight hours, this occurrence was rare. (Cultures of a mixture of the organs were positive up to and including twenty-five days. Blood cultures were positive up to forty-eight hours.)

Reaction of Host.—1. Tubercle formation: The paucity of tubercle bacilli was concomitant with a small number of tubercles. The early leukocytic response was infrequent. The Kupffer cells or histiocytes which contained bacilli as soon as one and a half hours after inoculation responded by a proliferation, forming small clusters. Small nodules were thus formed, composed only of histiocytic cells and an occasional lymphocyte (fig. 8). Nodules composed of granule-containing bacilli, lymphocytes and histiocytes were also found but were not numerous. By the seventh day the tubercles were composed of multinuclear giant cells containing acid-fast granules and rare bacilli, surrounded by epithelioid cells, granulocytes and lymphocytes (fig. 9). From the seventh to the ninth day there was an ever increasing proportion of lymphocytes at the expense of the histiocytes. The nodules became progressively smaller. At from twenty-five to fifty days the latter were composed of only a few histiocytic cells and lymphocytes (fig. 10). The former still contained a few acid-fast granules. Similar nodules could be found in the three month specimen. At fourteen months such nodules were entirely absent. Numerous control animals rarely showed a process similar to that described.

The tubercle in the liver differed from that in the lung in that it was smaller and often was composed from the beginning of proliferated histiocytic elements. Typical Langerhans giant cells were not found. On the other hand, the nodules persisted for a longer period than did those in the lung.

2. Reticulo-endothelial response: The reaction of the Kupffer cells as well as of the perivascular histiocytes was more prominent than the formation of tubercles. Swelling of the former cells occurred immediately after inoculation, and progressed as the time went on, as noted by phagocytosis of tubercle bacilli and granules of various descriptions. These proliferations at times arranged themselves into small nodules (fig. 8), with or without tubercle bacilli forming the focus of reaction. The perivascular histiocytes also were active in the process, and the two types of cells appeared to be active long after tubercules and bacilli had disappeared. At fourteen months these cells were hyperchromatic and flat and showed a minimal amount of phagocytosis.

SPLEEN

Reaction of Tubercle Bacilli.—The disappearance of tubercle bacilli from the spleen was rapid. At three hours they were few and already acid-negative. They were found only rarely at sixteen hours, and after that the finding of bacilli was the exception.

Reaction of Host.—1. Tubercle formation: Tubercle formation was of rarer occurrence here than in the liver, although bacilli were frequently found in large numbers. An epithelioid tubercle was noted on the ninth day.

2. Reticulo-endothelial response: The follicles, which at first contained no germinal centers, began to show them after eight hours and continued to do so up to seven days. At nine days the secondary follicles were indistinct, and at twenty-four days, not found. Occasionally small centers were noted after one and

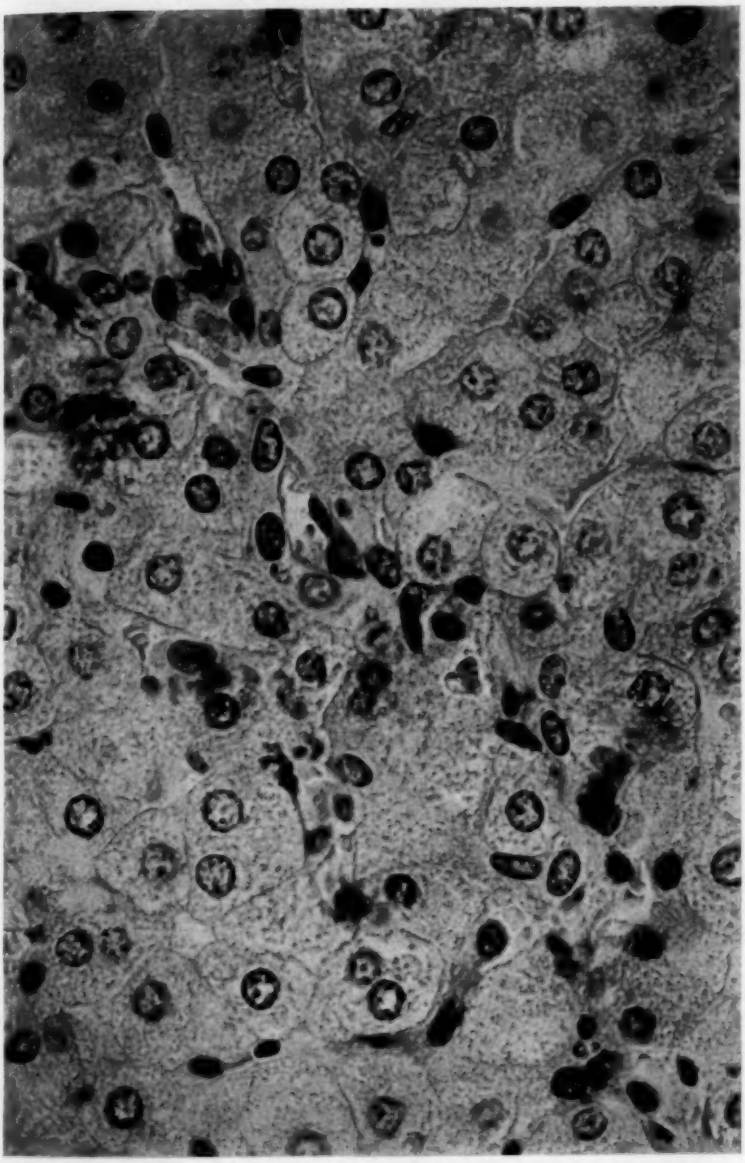

Fig. 8.—Liver sixteen hours after injection of bacilli; × 500: Proliferation of the Kupffer cells and grouping of these cells into small nodules. Some of the cells contain tubercle bacilli.

a half and three months. The proliferated centers were composed of reticulum cells as well as large and medium-sized lymphocytes. At times such proliferated areas were noted eccentric to the central artery. The endothelioid cells lining the sinuses showed active phagocytosis of both blood cells and tubercle bacilli. Blood pigment was found in increasing quantities after eight hours and until forty-eight hours, after which time there were only moderate amounts. The reticulum cell

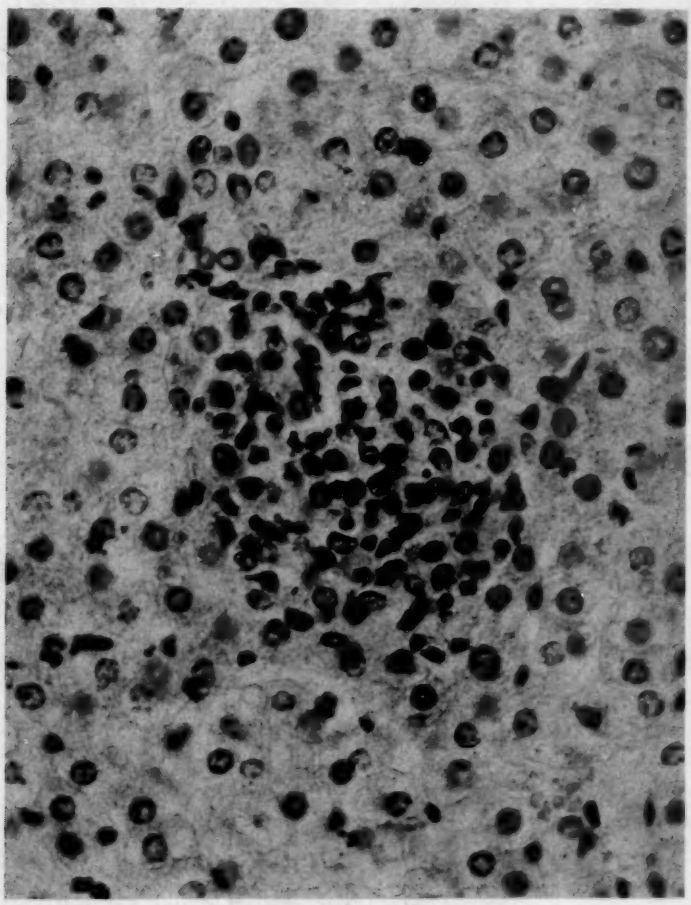

Fig. 9.—Liver seven days after injection of bacilli; × 500: A miliary tubercle composed of epithelioid cells and a few granulocytes. In the periphery lymphocytes can be detected.

activity was similar to the general one. In the fourteen month animal there was some evidence of increased fibrous tissue.

LYMPH NODES

Reaction of Tubercle Bacilli.—Of the lymph nodes studied, none revealed any striking attraction for the tubercle bacilli. The organisms were found in the

intrapulmonary nodes three and sixteen hours after inoculation and in a hilar gland after nine days (fig. 5 *B*). In the latter node many granular forms appeared. An occasional bacillus was found in a posterior tracheal lymph node twenty-four days after inoculation. Multiplication of organisms within lymph nodes was not noted.

Reaction of Host.—The formation of tubercles was not frequent (one instance, fig. 5 *B*). The question whether the perivascular and interstitial intrapulmonary lymphocytic accumulations and the proliferation of the germinal centers were results of infection by tubercle bacilli is difficult to answer, because such changes were found to a lesser or greater extent in adult controls. That proliferative changes of this tissue did occur as a result of the presence of the bacilli cannot

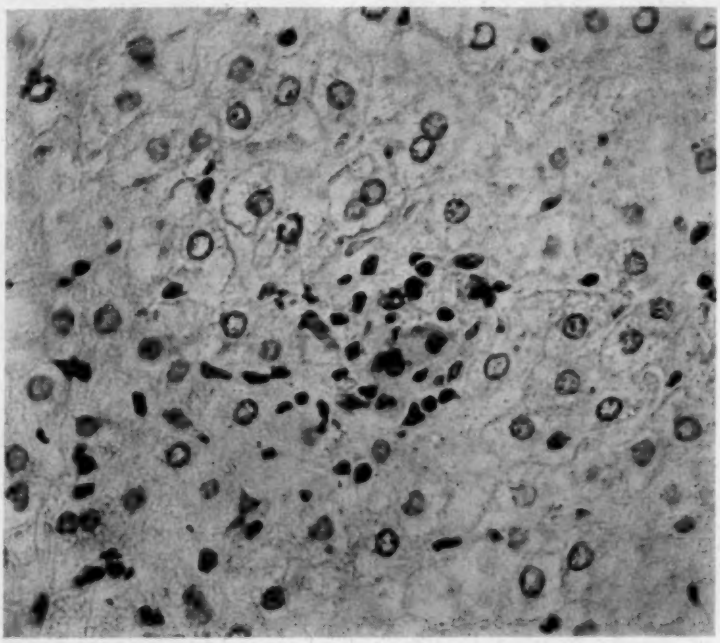

Fig. 10.—Liver one month and twenty days after injection of bacilli; × 500: A regressing tubercle showing a central giant cell surrounded by a few epithelioid cells and many lymphocytes.

be denied in some instances, especially if the organisms were found within granulocytes in the intrapulmonary lymph nodes.

In following guinea-pigs from birth to maturity there was noted a gradual increase in the intrapulmonary lymphoid elements. However, there was not a direct correlation between the lymphoid changes and age. The appearance of allergy did not seem to influence the lymphoid reaction.

KIDNEYS

Reaction of Tubercle Bacilli.—A few minutes after inoculation, acid-fast bacilli were already in the glomerular tufts, some free (20 per cent) and others within

granulocytes. These disappeared quickly, and after five hours acid-fast granules could be found within the interstitial histiocytes. Bacilli could not be detected from the second to the third day after inoculation, but in the seventh day specimen small bacillary clumps were noted, forming the centers of tubercles. Clumps of organisms were found up to fifteen days but were markedly decreased by the twenty-fourth day. After this time the organisms were extremely rare.

Reaction of Host.—1. Tubercle formation: Tubercle bacilli were found within the glomerular tufts, but the latter were not the usual site of tubercle formation. More commonly, tubercles formed beneath the capsule and adjacent to a glomerular tuft. Although the centers of the nodules were usually composed of granulocytes that had engulfed bacilli, not infrequently the tubercles were composed from the beginning of histiocytic elements exclusively. Regression of the tubercles followed in a manner similar to that described in foregoing paragraphs. No attempt at fibrosis or caseation was noted.

2. Reticulo-endothelial response: The cells of the reticulo-endothelial system that responded were the endothelium of glomeruli and of the afferent and efferent capillaries, the interstitial and the perivascular histiocytes (fig. 11). Almost immediately after the inoculation these cell groups displayed rounding and prominence. This prominence increased so that after five hours, notwithstanding the absence of noticeable bacilli, a piling up of the interstitial cells, especially of those of the cortex, occurred (fig. 12). The perivascular histiocytes, especially those of the perirenal fat tissue, were similarly affected. The activity of the interstitial cells suggested that these might be the source of the epithelioid cells of the tubercles. Nodules of these cells appeared without the presence of tubercle bacilli. After the tubercles became resolved by lymphocytic infiltration and decrease of epithelioid cells, focal areas of histiocytic accumulations were still noted up to one month and twenty days. In controls as well as in the two month and fourteen month animals such reactions were exceedingly rare.

HEART

Reaction of Tubercle Bacilli.—On the third day tubercle bacilli were found in clumps, and granules were not yet noticed. By the seventh day acid-fast and nonacid-fast granules appeared in large numbers, with only a few bacilli. By the twenty-fourth day only the granular forms were in evidence.

Reaction of Host.—1. Tubercle formation: The tubercle was noted in the epicardium or in the myocardium by the third day and was composed of a central area of degenerated pus cells and bacilli surrounded by a wide zone of epithelioid cells and lymphocytes. Giant cells were noted on the twenty-fourth day. Restitution resulting in fibrosis caused adhesion between the pericardium and the epicardium. The muscle cells seemed to enter into the epithelioid and giant cell formations.

2. Reticulo-endothelial response: This reactivity was almost as marked as that surrounding the tubercle itself despite the absence of tubercle bacilli. True, at times one found epithelioid cells at the periphery of the tubercle or even at a short distance from it containing acid-fast organisms or granules; yet this occurrence was localized. Epicardium, endocardium and myocardium seemed to take part in the reaction. The epicardium particularly showed after the third day a marked general histiocytic response (fig. 13). This reaction in the sixty-nine day specimen was replaced by a fibrotic one. The myocardial reaction was both interstitial and perivascular. Accumulations of histiocytes with caterpillar-like nuclear chromatin suggesting myocytes were noted in small groups simulating Aschoff

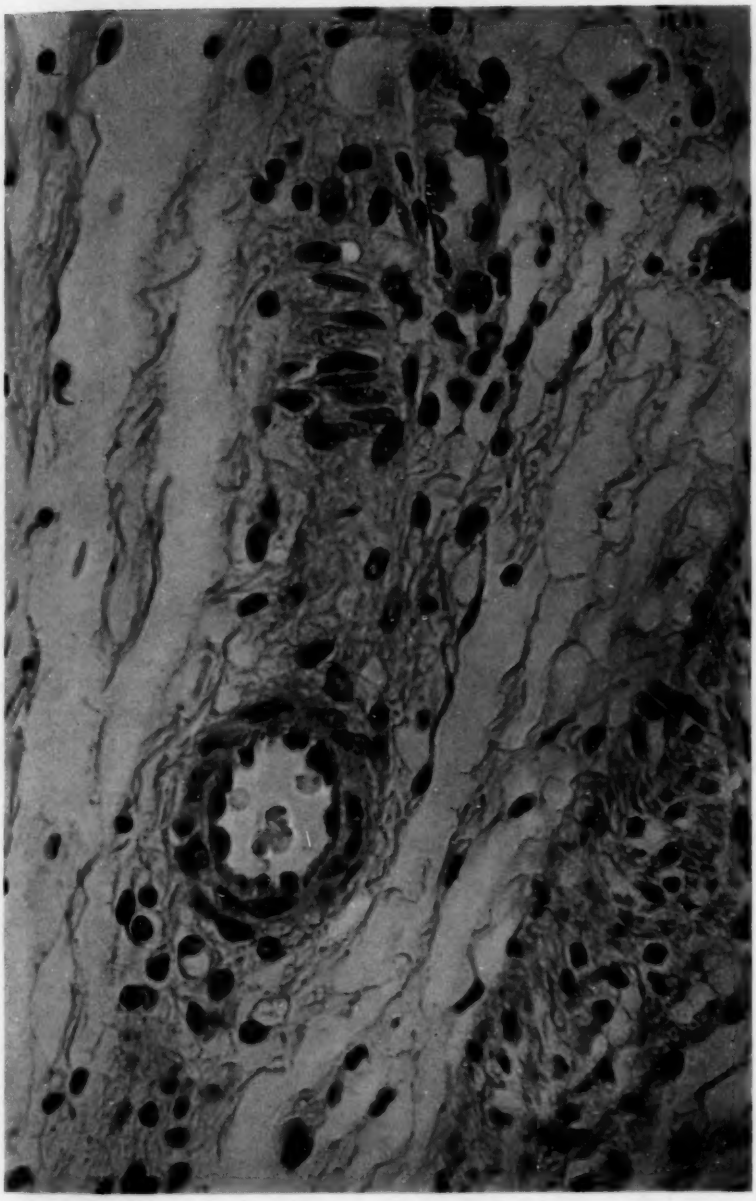

Fig. 11.—Kidney one and one-fourth hours after injection of bacilli; × 500: Peripelvic fat tissue showing marked histiocytic swelling and proliferation.

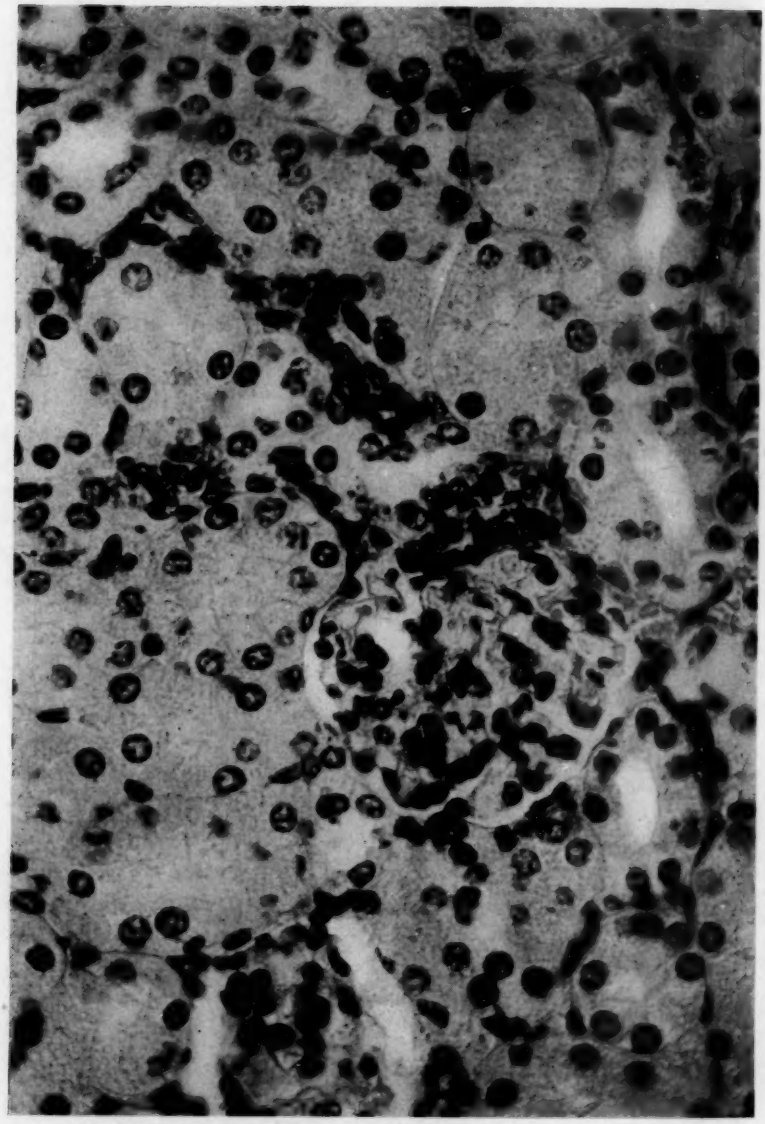

Fig. 12.—Kidney five hours after injection of bacilli; × 500: In the center of the field is a glomerulus showing swelling of the endothelium. The periglomerular capillary endothelium is prominent, and the perivascular histiocytes show rounding and proliferation. The interstitial histiocytes are prominent and show an increase in numbers, forming small nodules.

nodules. Similar changes were found up to one month but were entirely absent by the sixty-ninth day. The endocardial reaction was characterized by subendothelial proliferation of histiocytic elements, which at times assumed palisade-like arrangements (fig. 14). Definite nodules were formed with or without the infiltration of lymphocytes. In the sixty-nine day specimen such reactions were minimal.

BLOOD

That the blood cells reflect the tissue response in tuberculosis has long been suspected. Morriss and Tan [21] were the first to show that

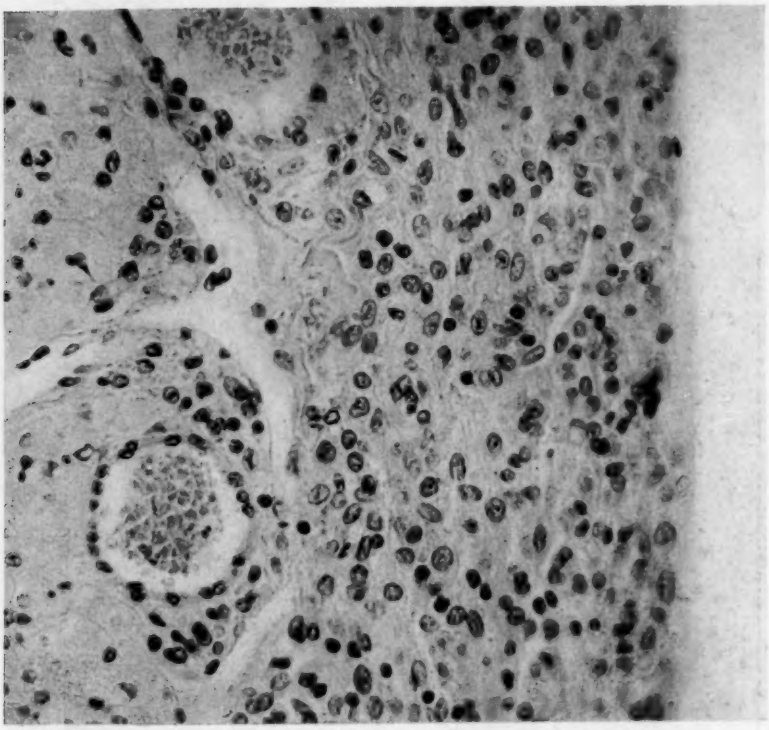

Fig. 13.—Epicardium three days after injection of bacilli; × 350: Extensive histiocytic proliferation in the absence of local tubercle bacilli.

in man the monocyte-lymphocyte (M/L) ratio of the blood could be used as an index of the progress of the disease. This work has been substantiated by Medlar [22] and his co-workers. Experimentally, both

21. Morriss, W. H., and Tan, S. H.: Am. Rev. Tuberc. **16**:729, 1927.

22. Medlar, E. M.: Tubercle **9**:218, 1928.

the bovine and the human type of tubercle bacilli caused changes in the circulating blood by which the progress of the disease could be adjudged (Camp, Sutton, Tompkins and Cunningham,[23] de Sanctis,[24] Birkhaug [25]).

Fig. 14.—Endocardium three days after injection of bacilli; × 350: Subendothelial histiocytic proliferation. Note the arrangement of these cells perpendicular to the surface endothelium.

23. Camp, W.; Sutton, F. H.; Tompkins, C. H., and Cunningham, R. S.: Am. Rev. Tuberc. **18**:462, 1928.

24. de Sanctis Monaldi, T.: Ann. Inst. Pasteur **44**:71, 1930.

25. Birkhaug, K. E.: Ann. Inst. Pasteur **51**:428, 1933.

The general impression is that the monocytes are related to tubercle formation and that an increase in their number in the blood marks the progression of tubercle formation (Cunningham,[17a] Doan and Sabin [26]). The leukocytes are an index of suppuration and abscess formation (Medlar [22] and Oatway [27]), and the lymphocytes supposedly indicate healing and repair (Murphy,[28] Medlar,[22] Oatway [27]). Briefly, then, an increase in the number of lymphocytes and a decrease in that of monocytes would cause a reduction of the M/L ratio and thus would indicate a favorable prognosis, while contrarily, the prognosis is poor.

Whether or not this holds true with BCG as applied clinically is not settled. Pittaluga [29] in one hundred vaccinated infants found a monocytic rise between the fifth and sixth day and in the period from the second to the fourth week, the rise being as high as 15 to 16 per cent. This was not substantiated by Doan and Moore,[30] Dolgopol and Stitt [31] and Baer,[32] who administered BCG orally, subcutaneously or intracutaneously. They found variations in the monocytes which they felt were within the normal limits (Forkner [33]). Pittaluga [29] criticized this work, especially that of Dolgopol and Stitt [31] and that of Forkner [33] in that the cases were too few. The final solution to the problem is still in the offing.

Experimentally, de Sanctis,[24] after injecting massive doses (30 mg.) of BCG intravenously in guinea-pigs, found an increase in the number of granulocytes and monocytes and finally in that of lymphocytes. (See Camp, Sutton, Tompkins and Cunningham [23] for human tubercle bacilli.) The following work was undertaken in an attempt to correlate the responses of the blood and the tissues.

Procedure.—The animals described histologically at regular intervals in the foregoing sections were followed first daily, then weekly and finally monthly by total and differential counts of the white blood cells. The guinea-pigs were bled from the ear, and the blood smears were stained by Wright's method. On the average, 200 cells were counted for each slide.

Results.—Normally the variations in the blood counts on guinea-pigs were found to be excessive, but for a given animal a greater stability was noted, especially in the relative proportions of cells. Total white blood counts varying from 3,000 to 10,000 were no uncommon. As a rule, in the controls, as well as in the experimental animals, before inoculations the lymphocytes constituted from 50 to 70 per cent of the total count, and the leukocytes, from 30 to 50 per cent. The

26. Doan, C. A., and Sabin, R. E.: J. Exper. Med. (supp. 3) **52**:113, 1930.
27. Oatway, W. H., Jr.: Am. Rev. Tuberc. **29**:73, 1934.
28. Murphy, J. B.: Lymphocyte in Resistance to Experimental Tuberculosis, Monograph 21, Rockefeller Institute for Medical Research, 1926, pt. 7.
29. Pittaluga, G.: Ann. Inst. Pasteur **49**:750, 1932.
30. Doan, C. A., and Moore, D. M.: Am. Rev. Tuberc. **23**:409, 1931.
31. Dolgopol, V. B., and Stitt, R. H.: Am. Rev. Tuberc. **26**:304, 1932.
32. Baer, H. D., quoted by Dolgopol and Stitt.[31]
33. Forkner, C. E.: Bull. Johns Hopkins Hosp. **95**:75, 1929.

monocyte count varied from 50 to 400 cells per cubic millimeter. Eosinophil and basophil counts were made but not recorded, because they did not enter into this study. The M/L ratio as seen in the normal animals rarely went above 0.1, the average being below that. Very shortly after intracardiac injection of BCG the total number of white blood cells was markedly increased. The granulocytes benefited almost entirely from this elevation. This condition remained for from eight to twenty-four hours, and then a downward trend began. The number of monocytes during this time was not appreciably altered, and that of lymphocytes decreased. At from forty-eight to seventy-two hours the granulocytes usually returned to within normal proportions, whereas the lymphocytes began to mount. The normal precedence of lymphocytes over polymorphonuclear leukocytes

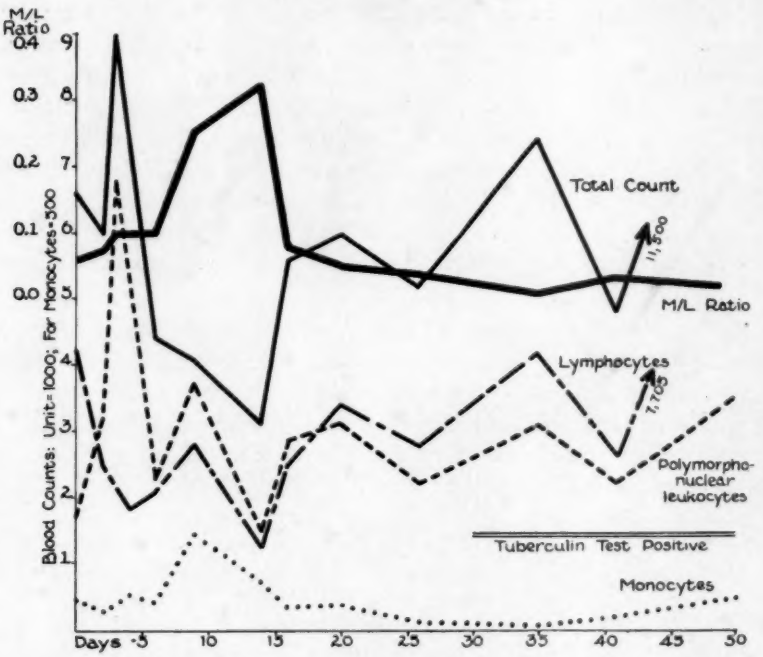

Fig. 15.—A representative blood picture in an animal receiving BCG intracardially.

recurred between sixteen hours and sixteen days and usually was established by the end of the first or the beginning of the second week. Vacillations after this reversion to normal occurred but were not common. The monocytes did not begin to rise appreciably until the third day, were usually at their peak from the seventh to the fifteenth day and receded to within normal limits by the end of the first month. The M/L ration followed an almost similar course (fig. 15). Tuberculin affected the blood counts only of certain animals. The alterations due to tuberculin, which were especially pronounced after allergy set in, were not constant. In one guinea-pig in which Koch's phenomenon had occurred an increase in the number of monocytes was manifest.

SUMMARY OF THE RELATIONSHIP BETWEEN TISSUE AND BLOOD RESPONSES

The increase in the number of leukocytes in the blood was transmitted and seen in the tissues. The increase in the tissues was not of long duration, and indeed after from eight to twenty-four hours a downward trend was noted. Swelling and proliferation of the histiocytic cells of the tissues began almost immediately, and by the third hour the cells were already seen to phagocytose granulocytes, especially at the sites of tubercle formation. This progressed at an increasing rate. The response of the reticulo-endothelial system was also generally characterized by a swelling of the septal cells of the lungs, the Kupffer cells of the liver, the perivascular histiocytes of the lungs, liver and kidneys, the endothelial cells of the glomeruli and the periglomerular capillaries of the kidneys, and the endothelial cells of the splenic sinuses and the reticulum cells of the spleen. The number of monocytes in the blood, however, did not increase appreciably during the early response of the reticulo-endothelial system. The cells of the aforementioned system entered into the formation of the tubercle, while the monocytes, the number of which increased definitely only after the third day, lagged behind the appearance of histiocytic elements in the tuberculous nodules. Only when the tubercle was fully formed (at from seven to fifteen days) was the number of the monocytes appreciably increased in the blood.

With regression of the tubercle as well as of the reticulo-endothelial response the number of monocytes in the blood decreased while that of lymphocytes increased. The lymphocytic increase in the tissues was reflected in the blood at this time, namely, at the end of the first or the beginning of the second week. As the tubercles receded the number of lymphocytes contained in them increased, and by the end of the first month the tubercle had practically become absorbed. The number of lymphocytes in the blood at this time was usually far above that of granulocytes, and the M/L ratio had assumed a normal value.

In the animal in which old tuberculin called forth Koch's phenomenon an increase in the number of histiocytes in the blood was manifest. At fourteen months the changes in both the tissues and the blood had given place to a return to the normal limits.

GENERAL COMMENT

The response of the host to tubercle bacilli depends on the state of the former (particularly as regards the organs involved) and the number and the virulence of the latter. In his recent work Vorwald [17] accepted the Huebschmann phenomenon of exudation as being the earliest response in which leukocytes are seen in large numbers. This author reported the changes in the lungs only of rabbits and used large doses of

virulent human organisms (0.1 mg. injected intravenously). Krause,[7] on the other hand, stressed the lymph follicle of the lung as a focus susceptible to the development of the tubercle in which the focal reticulum cell response is primary (proliferative phenomenon) as did Joest and Einhoff, also Zeyland.[34] Cunningham,[17] Doan and Sabin,[26] and Forkner [33] ascribed the development of the entire tubercle to an exudative process, and they expressed the belief that not only the leukocytes but also the epithelioid cells are derived from the blood. Thus one has two schools of thought to consider—the one holding that the process is humoral or exudative, and the other that it is tissue or proliferative.

By employing avirulent tubercle bacilli (BCG) with which the general reaction is less marked than with the virulent type, a better understanding of the rôle of the blood and tissue was had because the reactions were more readily dissociated. In the lung the blood granular leukocytes which had phagocytosed tubercle bacilli formed the focus of the early tubercle in almost every instance. Rarely were bacilli found within germinal centers of intrapulmonary lymph nodules, but here, too, the organisms were intracellular (within polymorphonuclear leukocytes). There was in such lymph nodes a proliferation of the reticulum cells of the germinal centers. Nodules were at times formed by epithelioid cells which had phagocytosed tubercle bacilli; such nodules called forth mainly a histiocytic type of cell. In the liver and in the kidney, where cells of the reticulo-endothelial system had phagocytosed bacilli as early as three hours after inoculation, such foci did not attract granulocytes but rather focal histiocytes, and the result was a purely histiocytic tuberculous lesion (see also the statement concerning lymph follicles of the lung). It seems, then, that if bacilli are first engulfed by granulocytes, as occurs within the blood, their presence in the tissues attracts by chemotactic action other granulocytes, whereas if organisms are first phagocytosed by focal histiocytes such foci call forth a local histiocytic response. The number of bacteria also plays a rôle; large numbers are associated with granulocytes and small numbers with histiocytes. In addition to this focal response of the host, there appeared also a general response of the reticulo-endothelial system, which was initiated soon after the introduction of the bacilli and continued during and following the formation of tubercles. The blood monocytes, on the other hand, did not increase in number appreciably until the period from the third to the seventh day, when the tubercles were already well along in their development. Not infrequently, little or no increase in the number of these cells occurred in the face of tubercle formation. The increase in the number of monocytes in the blood then lagged behind the formation of tubercles.

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34. Zeyland, J.: Am. Rev. Tuberc. **32**:588, 1935.

The regression of the tubercles was allied with an increase in the number of lymphocytes in the latter and a corresponding increase in the blood. The epithelioid cells decreased in number gradually, and finally complete restitution took place. The complete disappearance of the lesions took place in from one to three months, depending on the animals and the organs observed. Fibrosis was rarely seen and thus was not an essential in the healing of these tubercles. It is more likely that a fibrous capsule, although preventing the spread of the organisms, also restricts their being acted on by the host, and may account for the dormant and viable state of bacilli in well encapsulated tubercles.

The general sensitivity as noted by the test with old tuberculin did not become manifest until from the fourth to the seventh week, long after the tubercle had formed and in many instances when it was practically absorbed. The reticulo-endothelial activity as seen by the accumulations of focal histiocytes, phagocytosis, vacuolation and other changes was discernible long before the appearance of allergy. This same system could be stimulated to a more marked activity in certain animals by the injection of old tuberculin.

What is the mechanism responsible for the generalized response of the reticulo-endothelial system? Whether this be the result of a stimulation by way of the sympathetic nervous system (Bogendorfer [35]) or the result of a general diffusion of submicroscopic forms cannot be answered with assurance. The latter view is favored here because in another publication [36] it has been shown in the aforementioned animals whose organ cultures were negative (also microscopically negative) that serially inoculating said organs resulted in the recovery of acid-fast bacilli after the third to fourth generation. (All organ inoculations were made intraperitoneally.) According to Fontes,[37] Calmette, Valtis [38a] and Boquet, Négre and Valtis,[38b] the filtrable forms of the tubercle bacilli may be recovered only after serial inoculations as described.

The similarity between tubercles formed by virulent and those formed by avirulent tubercle bacilli is great, but there are many points of divergence. The nodules formed by BCG may be initiated with the accumulation of granulocytes or may be proliferative from the beginning through the appearance of epithelioid cells. There is rarely the sharp demarcation of polymorphonuclear leukocytes, epithelioid cells and lymphocytes found as a rule in tubercles formed by virulent organisms.

35. Bogendorfer, quoted by Jaffé.[13]

36. Rosenthal, S. R.: Am. Rev. Tuberc., to be published.

37. Fontes, A.: Mém. do Inst. Oswaldo Cruz **2**:141, 1910.

38. (*a*) Valtis, J.: Compt. rend. Soc. de biol. **90**:19, 1924. (*b*) Boquet, A.; Négre, L., and Valtis, J.: ibid. **94**:235, 1936.

Giant cells of the Langerhans type are rare, and caseation and necrosis are absent with BCG. With the latter, fibrosis is minimal, and restitution is complete.

The most significant differences, then, are that BCG does not cause the severe damage to tissue that leads to necrosis or caseation, and that the tubercle formed regresses completely usually without fibrosis or calcification.

Grossly, after intracardiac injection of BCG miliary tubercles about 1 mm. in diameter appeared in the lungs, liver, kidneys, spleen and lymph nodes in the order named. These nodules were found after the first to the second week and were exceedingly rare after the first month. The lesions were yellowish gray and rarely presented zones of hyperemia about them. These tubercles did not produce noticeable scars or calcific nodules. In contradistinction, the miliary nodules produced by virulent organisms are usually much larger and show definite perifocal zones of hyperemia. Not infrequently, these nodules become surrounded by a fibrous, hyaline or specific capsule, and the central portion shows softening.

The lymph nodes, especially the posterior tracheal, the mesenteric and the ileocecal nodes, following injection of BCG became enlarged as a result of swelling and hyperemia during the first week, and many continued to be enlarged (especially the posterior tracheal nodes) for several months. They rarely showed tubercles that were discernible grossly. The tubercle formation, caseation, fibrosis and calcification in the lymph nodes following the injection of a virulent strain of tubercle bacilli are well known.

GENERAL SUMMARY

Following the intracardiac injection of avirulent tubercle bacilli (BCG) there developed not only a specific reaction with tubercle formation in various organs but a generalized histiocytic response manifest most prominently in the septal cells of the lungs, Kupffer cells of the liver, endothelial and reticulum cells of the kidneys and spleen and histiocytes of the endocardium and epicardium.

This generalized response of the reticulo-endothelial system is ascribed to submicroscopic forms of the tubercle bacilli (BCG) as shown by cultural studies.

The formation and regression of the tubercle have been traced. The number of bacilli and the organs involved determined to a large extent the proliferative or exudative character of the nodule. The infiltration of the tubercle by lymphocytes was associated with the healing of the process.

The changes in the blood reflected those of the tissues.

The fate of the bacilli (BCG) differed in the various organs.

HEPATIC CHANGES ON INJECTION OF SODIUM DEHYDROCHOLATE IN CATS WITH TOTAL BILE STASIS

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Considerable information has been obtained regarding the changes that occur following obstruction to the outflow of bile, but the pathogenesis of many of these phenomena is still open to speculation. When the common bile duct is occluded the intraductal pressure increases until it reaches a point at which active secretion by the hepatic cells is believed to cease. However, it is probable that the contents of the biliary conducting system are subsequently constantly being reabsorbed into the lymph and blood streams and replenished through excretion by the hepatic cells. Certain of the changes that develop in the liver are commonly attributed in part to the extravasation of bile following the increase in intraductal pressure (regressive changes and proliferation of small bile ducts and associated connective tissue). It is also commonly believed that the bile acids constitute the toxic factor responsible for the initiation of these processes, although it is possible that some other constituent of stagnant bile possesses irritant properties not exhibited by other constituents of normal bile. The fact that white bile is generally regarded as nontoxic does not completely contradict this hypothesis. However, the problem is complicated by the fact that the formation of bile acids begins to diminish shortly after the induction of total bile stasis, these substances disappearing from the organism after a variable but relatively brief period. In view of the fact that bile acids under normal conditions constitute a potent stimulus to the secretion of bile, increasing both its volume and secretory pressure, and in view of the coincident changes in intraductal pressure and bile acid formation during biliary stasis, an investigation of the influence of bile salts administered during total stasis should prove interesting. The majority of bile salts are too toxic to be employed in this connec-

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tion, but sodium dehydrocholate,[1] which is a physiologically active bile salt preparation, is suitable for use in the practical investigation of this problem. In the present study this substance, which is regarded pharmacologically as of relatively low toxicity, was administered intravenously to cats with total bile stasis produced by ligation of the common bile duct. The rather marked lesions which were observed in the kidneys of these animals have been described in a previous communication.[2]

MATERIAL AND METHODS

Fifty-one cats weighing from 1.3 to 4.3 Kg. were employed. They were maintained on a diet of fresh raw scrap meat and milk. They were divided into two groups, twenty-two serving as controls and twenty-nine being subjected to ligation of the common bile duct.

Animals with Total Bile Stasis.—In twenty-nine cats the common bile duct was ligated close to the duodenum at laparotomy under ether anesthesia. After twenty-four hours of biliary stasis, sodium dehydrocholate (0.2 cc. per kilogram of body weight in 20 per cent solution) was injected into a vein of the leg. Individual animals received from one to fifteen injections during periods of stasis of from two to eighteen days. The injections were made at the rate of one every twenty-four hours except in a few animals with prolonged stasis in which a few injections were made at intervals of forty-eight hours. The animals were killed by bleeding under light ether anesthesia twenty-four hours after the last injection, except two on which autopsy was performed four and eight hours, respectively, after the last injection. At this time estimations were made of the bromsulphalein retained in the blood thirty minutes after injection of 2 mg. per kilogram and of the bilirubin in the serum (van den Bergh). This series includes no animal in which there was any evidence of accidental infection or of patency or of reconstruction of the common duct or of entrance of bile into the intestine through anomalous or accessory ducts.

Control Animals.—Sodium dehydrocholate (0.2 cc. per kilogram in 20 per cent solution) was injected into a leg vein in sixteen cats at twenty-four hour intervals over periods of from one to seventeen days. Each animal was killed by bleeding under light ether anesthesia twenty-four hours after the last injection. Three cats were killed in a similar manner two, three and four days, respectively, after a single injection. Three additional cats were killed one, two and three days, respectively, after a single injection of sodium dehydrocholate in similar dosage into the portal vein, exposed at laparotomy under light ether anesthesia.

Pieces of tissue were fixed in a solution of formaldehyde (1 : 10) or a solution of formaldehyde plus Zenker's solution. Some were subsequently frozen, cut and stained with nile blue sulfate and scarlet red, and the remainder were blocked in paraffin, cut and stained with hematoxylin and eosin and Mallory's connective tissue stain. Frozen sections (from 8 to 25 microns thick) were examined under crossed Nicol prisms for doubly refractile material.

1. Sodium dehydrocholate is available in the form of decholin sodium (obtainable from Riedel-de Haen, Inc., New York, which has been accepted by the Council on Pharmacy and Chemistry of the American Medical Association). The decholin sodium employed in the present study was supplied by Mr. Paul de Haen.

2. Stewart, H. L., and Cantarow, A.: Arch. Path. **20**:866, 1935.

GROSS CHANGES IN STASIS

Proximal to the ligature the extrahepatic ducts underwent progressive dilatation with thickening of their walls which, although variable in degree, was in general more or less proportional to the duration of stasis. These changes were essentially the same as those noted in animals with uncomplicated stasis, although in a few instances the distention of the ducts appeared to be increased out of proportion to the duration of stasis. In the cats in which cholecystectomy had not been performed the gallbladder became distended, its walls thickened and the mucous membrane hyperplastic, with papillary projections of varying size, some measuring as much as 1 to 2 mm. in thickness. In one animal (cat 23, stasis thirteen days, nine injections) the gallbladder, while markedly distended, was not tense, and portions of its wall were greatly thinned and dark, the mucous membrane being entirely absent in the areas of involvement, which occurred in portions of the organ not in direct contact with the liver.

The bile in the gallbladder was dark green in every instance and was usually thick and tarry, even in the early stages of stasis. The bile in the extrahepatic ducts varied considerably in color and consistency throughout the course of the experiment; these characteristics bore no apparent relation to the duration of stasis or to the presence or absence of the gallbladder, which occasionally contained bile of an entirely different appearance from that present in the ducts. Although very pale, thin bile was observed in a number of cats, white bile was present in only one (stasis five days, four injectons).

The liver was usually somewhat enlarged and smooth, and the color varied from pale yellow to deep brown; in a few cats, in the later stages of the experiment, the liver was slightly granular and dark green or brown. The hepatic markings beneath the capsule and on the cut surface were almost invariably accentuated, consisting of a central deep red area surrounded by well demarcated yellow and brown zones. In a few animals there were small localized areas of fibrinous exudate on the surface. On section the intrahepatic ducts showed varying dilatation and thickening consistent with the duration of stasis and usually contained bile similar in appearance to that in the extrahepatic ducts. In a few ducts the bile was pale and turbid, and smears from it revealed numerous leukocytes. The progressive connective tissue proliferation and inflammatory cell infiltration was evidenced grossly by stellate gray markings in the periportal areas. In four animals (cat 17A, stasis nine days, seven injections; cat 22, stasis thirteen days, eleven injections; cat 24, stasis fourteen days, twelve injections; cat 28, stasis eighteen days, fifteen injections) the surface of the liver presented areas of dark brown hyaline necrosis, sharply defined, slightly depressed and firm. These were most numerous in the region of the hilus; they tended to extend and widen toward the free margin of the liver; in one animal they were widely distributed throughout the parenchyma. In these areas the external lobular markings were indistinct or absent, the pale fine stellate periportal markings and thickened dilated ducts standing out in sharp contrast against the dark background. On section these lesions were found to be distributed along the course of large intrahepatic ducts, which were enormously dilated and invariably contained white bile, although the remainder of the duct system contained dark green or brown bile. The cut surface of these areas showed an absence of lobular markings and considerable parenchymal atrophy. In one animal (cat 23, stasis thirteen days, nine injections), a sharply demarcated pale area of infarction was present in the tip of one lobe.

MICROSCOPIC CHANGES IN STASIS

Pigmentation.—The intensity of pigmentation varied considerably throughout the period of the experiment. In morphology and distribution it was essentially the same as that previously noted in simple stasis and bore no constant relation to the duration of obstruction or to the number of injections of dehydrocholate. The intensity of pigmentation was not consistently related to hepatic cell degeneration and necrosis in the inner portion of the lobule. In one animal in this series (cat 20, stasis eleven days, nine injections) the pigmentation was more intense than has been observed by us in any animal with simple stasis. In one animal (cat 27, stasis eighteen days, fifteen injections) an unusual lesion, somewhat resembling the pigmented nodular lesion of stasis,[3] was observed in relation to the large intrahepatic bile ducts. The involved areas were pale and the hepatic cell cords had disappeared, resulting in approximation of the sinusoids and Kupffer cells. Changes were noted which suggested that connective tissue proliferation increased progressively with obliteration of the capillaries, resulting in the formation of an area of fibrous tissue studded with small pigmented nodules.

Regressive Changes.—With certain noteworthy exceptions the regressive changes were of the nature of those previously observed in simple stasis,[4] i. e., diffuse and sporadic necrosis, and degeneration and necrosis of hepatic cells in the inner portion of the lobule, about the radicles of the portal vein and beneath the capsule of the liver. As in cats with simple stasis, the severity and extent of these regressive lesions varied considerably in individual animals at different periods of biliary obstruction. However, a distinct difference was noted in the appearance of the hepatic parenchyma at comparable stages in the two groups of animals, which was apparently dependent on the persistence of stainable lipid and the relatively marked activity of regeneration in the animals receiving hydrocholate. Throughout the period of observation, particularly in the later stages, the progress of the degenerative changes in the periportal areas was modified by the simultaneous development of large pale hepatic cells, possibly newly regenerated; the resulting combination of regressive and regenerative changes constituted a morphologic picture which differed strikingly from that presented by the compressed, atrophied cells in this situation during simple stasis. In the inner portion of the lobule also this relatively marked activity of regeneration was evidenced to a greater degree than in uncomplicated obstruction, the narrow cords of small flat newly regenerated hepatic cells in this area frequently presenting little or no evidence of degeneration.

Throughout the liver, in many portions of the lobule, particularly with stasis of from seven to thirteen days' duration, atrophied, compressed, degenerated and necrotic hepatic cells, at times intensely acidophilic, alternated with large, swollen pale hepatic cells. The latter were in some instances possibly newly regenerated cells, and in others their appearance was dependent on a high content of lipid, but in some instances the factor responsible for their swollen character could not be determined. Occasionally, the coincident presence of small newly regenerated cells in the inner portion of the lobule, atrophied and degenerated cells and large, swollen cells resulted in a distinct diminution in the size of the lobule, with approximation of the central vein and the portal radicles. These changes resulted at times in a change in the regular cordlike arrangement of the hepatic cells, the

3. Stewart, H. L., and Lieber, M. M.: Arch. Path. **19**:34, 1935.

4. Cantarow, A., and Stewart, H. L.: Am. J. Path. **11**:561, 1935. Stewart and Lieber.[3]

usually straight rows of which were twisted into S-shaped curves. In one cat (9) many hepatic cells were irregularly shaped and were loosened from their reticular attachments, a phenomenon frequently noted by us following biliary decompression.[5] In general, the hepatic parenchyma was surprisingly free from extensive regressive changes of the varieties mentioned as occurring at comparable stages of uncomplicated stasis.

A peculiar regressive change, not noted by us in animals with simple stasis, was observed in eight of twenty-one animals which had stasis of from two to eleven days' duration and which received from one to ten injections of dehydrocholate. Groups of from two to fifteen, usually from three to eight, hepatic cells were involved in this process: the cytoplasm became pale, finely reticulated or vacuolated, and the nucleus became pale and swollen. Frequently this change progressed until the nucleus completely disappeared, the cytoplasm crumbled and disintegrated and the nuclear and cellular outlines were lost, leaving round or oval vacant spaces with irregular margins composed of the faded cytoplasm of the still viable adjacent hepatic cells. These areas varied considerably in number and distribution, being at times most numerous near the hilus and at times away from the hilus; as many as twenty were observed in a single lobule, and when present in large numbers they imparted a rather characteristic "moth-eaten" appearance to the hepatic parenchyma (fig. 1). In one animal some of these areas were infiltrated by degenerated inflammatory cells. In other cats, however, such cells were observed in groups of from eight to fifteen bearing no relation to the degenerative lesions either in occurrence or in distribution, and they were present in animals in which the latter process was absent.

Areas of hyaline necrosis were observed in the subcapsular region, sporadically throughout the lobule and in the periportal areas. In character, distribution and extent these lesions were comparable to those noted in animals with uncomplicated obstruction of equal duration, and the experimental period was not prolonged to the stage at which organization of the necrotic areas with marked fibrosis and compression and strangulation of the bile ducts would be expected to occur. In four cats (17A, 22, 24 and 28) hyaline necrotic lesions of a rather distinctive character were observed, the gross appearance of which has been described. This process, which was particularly marked about the larger intrahepatic bile ducts, involved cells singly, in groups of from three to ten or in extensive patches extending deep into the parenchyma. The cytoplasm of the affected cells was deeply acidophilic and homogeneous, and fusion of several cells was frequently observed; the nuclei were intensely basophilic, pyknotic or swollen and at times were completely disintegrated. In the larger areas of involvement the portal areas were necrotic, the sinusoids compressed and the blood vessels thrombosed, particularly the veins, and the venous thrombi occasionally showed evidence of organization. When superficial, the atrophy of involved areas of the parenchyma resulted in depression of the surface of the liver. These lesions somewhat resembled infarcts. Infarcts were present in typical form, however, in only one cat (23).

Although in a few cases there was some question of the possible relation of the small groups of necrotic hepatic cells to focal midzonal necroses, the latter were not observed in their characteristic form in a single animal.

Bile Duct Changes.—The dilatation of the larger intrahepatic and extrahepatic bile ducts which was consistently observed grossly was not as readily demonstrable

5. Stewart, H. L., and Cantarow, A.: Am. J. Digest. Dis. & Nutrition **2**: 101, 1935.

in histologic sections because of the collapse of this portion of the duct system when the increased intraductal tension accompanying total bile stasis was relieved. A striking difference from the condition of these ducts in simple stasis consisted in a marked infiltration of their walls by inflammatory cells, chiefly lymphocytes and plasma cells but also mononuclears and polymorphonuclear neutrophils and eosinophils; the latter tended to be scattered diffusely and were often degenerated, while the small round cells were frequently collected in nodules and were usually well preserved The proliferation of connective tissue in the walls of the ducts was of approximately the same degree as in uncomplicated bile stasis, but the proliferated tissue was very compact and rather vascular. Proliferation of the

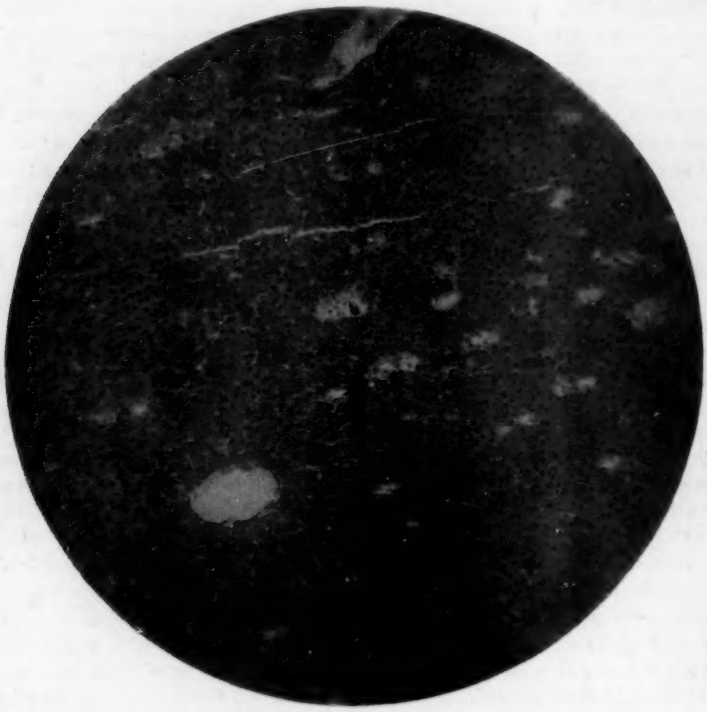

Fig. 1.—Section of liver showing "moth-eaten" areas of necrosis. Magnification about × 100.

mucous membrane with mitosis of the bile duct epithelium occurred as in simple obstruction. From the eleventh day on, regressive changes appeared in the proliferated mucosa, namely, hydropic swelling and desquamation, involving first the superficial lining cells and subsequently deeper layers of cells. These regressive changes were accompanied by an increase in the diameter of the lumen; the previously large, hypertrophic villi remained as short projecting spurs of mucous membrane, and the crevices between these villi frequently contained masses of inspissated pigment.

With a few exceptions the changes in the medium-sized ducts were essentially the same as in the larger ducts. In a few ducts the lining epithelial cells were pale,

swollen and vacuolated, and presented the rather characteristic appearance described by us [5] in animals following biliary decompression. Then, too, in some cats the inflammatory cell infiltration and fibrosis were more marked in the medium-sized ducts than in the larger ducts, the former frequently containing inflammatory cells within their lumens. This inflammatory process also frequently involved the stroma of the mucous membrane, the infiltrating cells being distributed diffusely or confined to single papillary projections of mucosa.

Proliferation of the smallest bile ducts gradually progressed during the first week, as in simple stasis. After the eighth day, with rare exceptions, the proliferation was much less marked than at corresponding periods of uncomplicated stasis. This difference was particularly notable after the thirteenth day. A striking feature of these proliferated ducts after the third day was that the great majority, and in some cats all, presented distinct lumens, with the cells somewhat flattened and hyperchromatic, in marked contrast to the solid, cordlike arrangement of these cells in simple stasis.

Throughout the course of the experiment after the third day the proliferated small ducts underwent regression, chiefly as a result of two processes: (1) accumulation of hyaline secretion and (2) crowding and displacement by adjacent newly regenerated hepatic cells. The process of disappearance through accumulation of hyaline secretion was essentially of the nature observed in simple stasis but appeared to be much more active under the influence of dehydrocholate. Regression of proliferated ducts as a result of displacement by regenerating hepatic cells, with consequent atrophy, constituted a striking point of difference from conditions observed in uncomplicated biliary obstruction, and closely resembled the mechanism of disappearance of these ducts during biliary decompression. The continuity of the ducts was interrupted by large pale apparently young hepatic cells, the small islands of duct cells frequently being eventually entirely replaced by the newly formed liver cells (fig. 2).

In contrast to simple stasis the proliferation of connective tissue in the portal radicles, about the smallest bile ducts, was never marked. This difference was especially striking after the second week, at which time the dehydrocholate-treated animals presented remarkably little evidence of connective tissue proliferation in this situation. Infiltration of the small portal radicles by inflammatory cells (polymorphonuclear leukocytes, mononuclears and small round cells) was most marked during the first nine days, being at times out of proportion to the degree of infiltration of the larger ducts.

Vascular Changes.—The vascular changes did not differ materially from those observed at comparable periods of uncomplicated stasis. In cases in which the cells in the inner portion of the lobule were of the narrow, flat variety the sinusoids appeared dilated but not unusually congested.

Regeneration.—Evidences of active regeneration of hepatic cells were observed throughout the course of the experiment; these consisted in binucleation, the formation of hypertrophic cells and hyperchromatic nuclei, active mitosis and the appearance of a flattened type of cell in the inner portion of the lobule and of pale swollen cells in the periportal areas. Mitotic figures were noted from the fourth to the fifteenth day, were at times present throughout the hepatic parenchyma including the subcapsular zone, were rarely observed in association with the flattened type of epithelium, and occurred with the greatest frequency about the small portal radicles, where they were usually associated with large pale hepatic cells which have been referred to previously in connection with the regression of the proliferated bile ducts. The small flat type of cell in the inner portion of the

lobule was observed at two and nine days, and the number appeared to increase progressively from the thirteenth to the eighteenth day. These cells were smaller and flatter than normal, with a small amount of cytoplasm and a relatively large hyperchromatic nucleus. Owing to the presence of an increased number of these small cells, hepatic cell nuclei were unusually abundant in the inner third of the lobule. In some cases, the narrow, ribbon-like cords of these cells were arranged in S-shaped curves, owing presumably to the presence of large numbers of swollen cells at the periphery of the lobules (fig. 3).

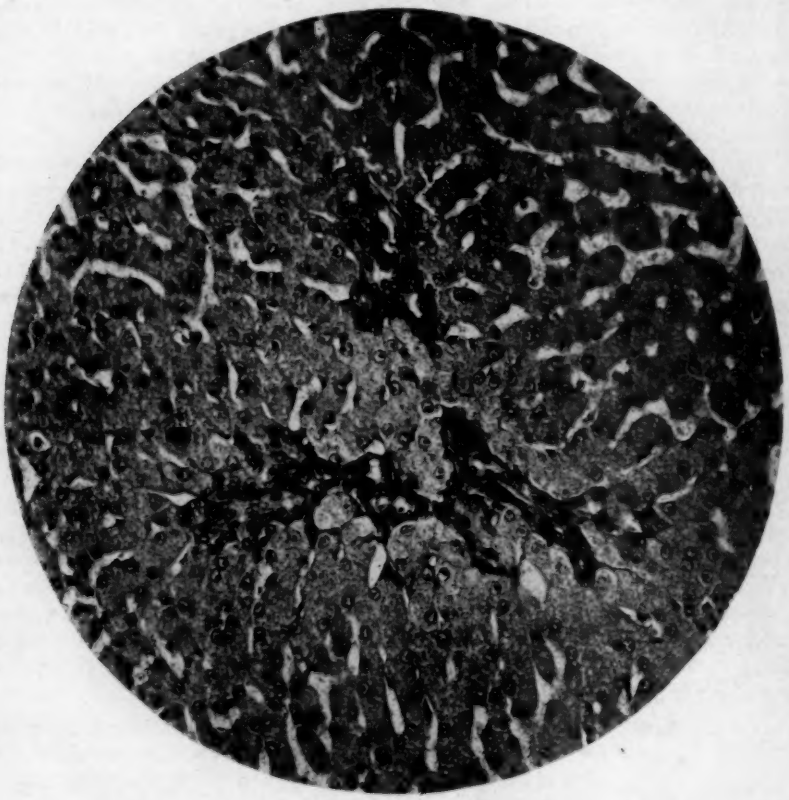

Fig. 2.—Section of liver (stasis eighteen days, fifteen injections) showing the extent of proliferation of bile ducts and connective tissue in small portal radicles. Note the large pale newly formed hepatic cells encroaching on proliferated ducts. Magnification about × 350.

Lipid.—The findings with regard to the presence and amount of stainable lipid and doubly refractile material are presented in detail in table 1. The quantity of stainable lipid in both hepatic and Kupffer cells was extremely variable throughout the entire period, bearing no apparent relation to the duration of stasis or to the number of injections of dehydrocholate. Stainable lipid was definitely absent from the hepatic cells in only one cat (23). Although in some cats the quantity

of this material diminished simultaneously in both hepatic and Kupffer cells, this relationship was by no means constant.

Doubly refractile material was present, usually in abundance, in the Kupffer cells throughout the period of the experiment. In two cats (9 and 21) the hepatic cells contained much more of this material than did the Kupffer cells; in six cats it was present in abundance in both situations, and in twenty cats the hepatic cells contained little or none (in seven, none). As in simple stasis, it was frequently

Fig. 3.—Section of liver showing central vein above and portal radicle below. The hepatic cells, particularly in the inner portion of the lobule, are of the flattened variety, the hepatic cell cords being narrowed and the sinusoids dilated without being congested. Magnification about × 200.

difficult to be certain as to whether the doubly refractile material was contained within Kupffer cells or was free within the sinusoids. There was no consistent relationship as regards amount and distribution between stainable lipid and doubly refractile material in either hepatic or Kupffer cells. This inconstancy as well as

the morphology and staining reaction of the stainable lipid and doubly refractile material was essentially the same as that observed in simple stasis.[6] Lipid material could almost never be demonstrated in the "moth-eaten" areas of degenerated and necrotic hepatic cells.

TABLE 1.—*Stainable Lipid and Doubly Refractile Material in the Livers of Cats with Total Bile Stasis Receiving Sodium Dehydrocholate*

Cat*	Weight, Kg.	Stasis, Days	Injections of Decholin Sodium	Stainable Lipid		Doubly Refractible Material	
				Kupffer Cells	Hepatic Cells	Kupffer Cells	Hepatic Cells
1	2.6	2	1	+	++++	++++	+
2	2.99	2	1	++	++	+++	0
3	2.47	2	1	++	++++	±	±
4	3.26	2	1	+++	++	++	0
5C	1.53	3	2	+	++++	++++	++++
6C	2.30	4	3	+	+++	++++	++++
7C	2.85	4	3	+	+	+++	±
8C	1.55	4	3	±	+	++++	++
9C	1.40	5	4	+	±	±	++++
10C	1.70	5	4	+	+	++++	±
11C	1.30	5	4	±	++++	++++	++++
12C	3.30	6	5	++	±	++++	++++
13	4.14	6	5	++++	++++	++	0
14C	2.24	7	6	+	±	++++	±
15C	1.70	7	5	±	++	++++	++++
16	1.33	8	6	++	±	++++	±
17C	1.84	9	8	++++	±	++	0
18	3.00	10	8	++++	++++	++++	++
19C	2.13	11	10	+++	+	++++	±
20C	2.49	11	9	++++	++++	++++	++++
21C	3.00	11	9	+	++++	+	++++
22	2.10	13	11	+	++++	++++	±
23	2.23	13	9	±	0	+++	0
24	3.22	14	12	±	±	+++	0
25	1.88	15	12	++	++++	++++	±
26	2.84	17	14	++	+++	++++	+
27L	2.07	18	15	++	+	++++	±
28	2.90	18	15	±	±	++++	0

* In this column C following the number indicates cholecystectomy and L ligation of the cystic duct.

TABLE 2.—*Concentration of Bilirubin in the Serum and Retention of Bromsulphalein*

Duration of Stasis, Days	Bilirubin, Mg.	Bromsulphalein, per Cent	Duration of Stasis, Days	Bilirubin, Mg.	Bromsulphalein, per Cent
2	0.0-2.8	0-100	10	4.72	60
3	0.48-7.46	0-100	11	7.34-8.6	80-100
4	1.8-5.75	30- 80	12	19	60
5	3.08-4.0	50- 90	13	0.2-19.5	10- 95
6	5.4-8.82	80-100	15	3.8-12.8	100
7	1.2-8.0	20-100	16	6.8	80
8	1.1-18	20-100	18	0.48-6.8	20-100
9	2.4-8.6	70-100			

CONCENTRATION OF BILIRUBIN IN THE SERUM AND RETENTION OF BROMSULPHALEIN

The observations with regard to the concentration of bilirubin in the serum and the retention of bromsulphalein were as shown in table 2.

6. Cantarow, A.; Stewart, H. L., and Lieber, M. M.: Arch. Path. **20**:535, 1935.

COMMENT

There is an extensive literature dealing with the toxicity of sodium dehydrocholate and with its effect on the volume and secretory pressure of bile, both of which are increased under its influence. Clara [7] has made a detailed study of the effect of this substance on the normal liver and bile ducts. His observations may be summarized as follows: Morphologic changes were present within eight to ten minutes after injection of this substance, were maximal after thirty to fifty minutes and then diminished but were still present at the end of twenty-four hours. These changes consisted essentially in dilatation of the canaliculi and smaller bile passages with flattening of the lining epithelium of the small intrahepatic bile ducts. These phenomena were regarded as directly related to choleresis, the stimulation of secretory activity being also evidenced by alterations in the glycogen content and by the appearance of secretion granules in the hepatic cells. The twenty-two animals employed by us as controls in the present study were examined under conditions comparable to those which existed in the group with stasis, and sections were prepared by the ordinary histologic technic. No significant morphologic changes were observed in the liver with the exception of occasional flattening of the duct cells, canalicular dilatation, narrowing of the hepatic cell cords in the inner portion of the lobule, dilatation of the sinusoids without congestion and in one cat (eight daily injections) the appearance of multiple, small rounded areas of fatty change, usually occupying less than one-fourth the diameter of the lobule. It may be noted that Clara found some evidence of hepatic damage after giving frequently repeated doses of dehydrocholate in amounts sufficient to produce toxic manifestations.

The administration of sodium dehydrocholate appeared to have a distinct effect on the livers of animals with total bile stasis. One feature of considerable interest was its influence on the regeneration of hepatic cells. The criteria that were employed as indexes of regenerative activity were (1) mitosis, (2) changes in the number and appearance of the nuclei in individual cells, (3) flattening of the cells in the inner portion of the lobule and (4) the appearance of pale swollen cells in the periphery of the lobules. There can be no doubt as to the validity of the first two of these criteria. Chiefly as a result of the observations of MacNider [8] the hypothesis has been promulgated that flattened cells in the inner portion of the lobule represent repair. As stated by MacNider,[8] "in the severe liver injuries the process of liver repair is effected by the formation of a type of liver cell which is atypical in

7. Clara, M.: Med. Klin. **30**:203, 1934; Ztschr. f. mikr.-anat. Forsch. **31**:193, 1932; **32**:521, 1933; **34**:379, 1933; **35**:1, 1934.

8. MacNider, W. deB.: Tr. A. Am. Physicians **49**:14, 1934.

its morphology, distinctly flattened, of a less specialized appearance and containing deeply staining nuclei, which in size are out of proportion to the cell cytoplasm which surrounds them." We have observed this type of cell in the inner portion of the lobule in cats with bile stasis following ligation of the common bile duct and single hepatic ducts as well as in the present series of animals which received sodium dehydrocholate, and we have observed it after biliary decompression. Our original opinion was that this change was atrophic in nature, but more recently a careful comparison of our material with the reported observations of MacNider has convinced us that the process is identical with that described by him. It is interesting to note that, as observed by MacNider,[8] mitotic figures were rarely seen in association with these flattened cells.

The interpretation of the pale swollen hepatic cells at the periphery of the lobules as being newly regenerated cells is based chiefly on observations made during biliary decompression.[5] During stasis the hepatic cells in the region of the small portal radicles gradually become atrophied and compressed by the distended and proliferating bile ducts. During decompression the hepatic cells in this situation become swollen, more numerous and more prominent, with a finely reticulated clear cytoplasm and hyperchromatic nuclei. Binucleation, multiple nuclei and active mitosis are frequently observed. These newly formed cells insinuate themselves among the proliferated ducts, compressing and crowding them aside; in some instances small islands of duct cells are apparently snared off and are surrounded by the regenerating hepatic cells. These changes during decompression eventually result in the restoration of an essentially normal parenchyma in this portion of the lobule. The appearance of this region in cats with total stasis receiving sodium dehydrocholate resembled that observed during decompression much more closely than that seen in uncomplicated stasis, and the assumption seems justifiable that these changes represent a regeneration of hepatic cells. The reason for this is not readily apparent, but the distinctly different behavior of these cells during simple stasis and stasis plus dehydrocholate makes it obvious that the latter substance supplied a potent stimulus to the regeneration of hepatic cells and repair of tissue that was absent during uncomplicated stasis. Another finding of considerable interest, indicating another point of resemblance between the effect of dehydrocholate and that of decompression, in addition to the extraordinarily active mitosis generally, was the appearance of mitotic figures in hepatic cells in the subcapsular zone, a phenomenon frequently observed by us [5] in decompression but never in uncomplicated stasis in the cat.

Another interesting feature was the fact that the proliferation of the smallest bile ducts and associated connective tissue was distinctly

less marked than in simple stasis after the eighth day, this difference being particularly striking after the thirteenth day. Furthermore, the extensive luminization of these proliferated ducts after the third day was in sharp contrast to the solid, cordlike arrangement of the duct cells during uncomplicated stasis. The reason for the inhibition of the proliferation of bile ducts and associated connective tissue and the persistence of lumens is not known, but here again the resemblance between the animals with stasis which were receiving dehydrocholate and those with biliary decompression is highly suggestive. The fact that the proliferation of bile ducts and of associated connective tissue was essentially of the same magnitude in individual animals suggests that these two phenomena are influenced by the same stimulating and inhibiting factors. Although no information is available regarding the effect of sodium dehydrocholate on the intraductal pressure during total stasis, there is certainly no reason to believe that this pressure is diminished under its influence. The fact that the proliferation of bile ducts and of associated connective tissue was distinctly diminished in these animals despite the persistence of total stasis appears to contradict the view that this proliferation is due solely to increased intraductal pressure. It also appears to contradict the hypothesis that this phenomenon is dependent on extravasation of bile into the adjacent tissue, although the possibility cannot be ignored that the bile is so diluted under the influence of the dehydrocholate that it is rendered less irritating. Furthermore, the fact that the newly proliferated bile ducts are well luminized in the dehydrocholate-treated animals may have some effect in diminishing the extent of extravasation of bile from the duct system into the surrounding parenchyma.

No definite statement can be made regarding the cause of the relatively marked infiltration of the intrahepatic bile ducts by inflammatory cells. This was much more marked than that noted during simple stasis, although the operative procedures employed were identical, and there is no reason to believe that infection was introduced subsequently. Furthermore, similar grades of inflammatory cell infiltration were observed in cats during biliary decompression.[5] The significance of this observation is doubtful.

With certain exceptions the hepatic parenchyma generally presented less evidence of regressive change than at comparable periods of uncomplicated stasis. As stated previously, this was due in large measure to the relatively marked activity of hepatic cell regeneration. In four cats (17A, 22, 24, 28), however, a distinctive process of hyaline necrosis was observed which in extent far exceeded any similar change noted by us in animals with simple stasis. The cause of this extensive lesion is not readily apparent. Its intimate association with vascular thrombosis indicates that it may constitute a process of infarction. However,

infarcts were present in typical form in only one animal in this series (cat 23). The significance of hepatic infarction following experimental ligation of the common bile duct is not clearly understood. One must give careful consideration to the possibility that it is the result of accidental vascular injury during the operation of ligation. It may be of interest to note here, however, that no instance of infarction has yet been encountered by us in animals after ligation of a single hepatic duct, a problem under investigation at the present time.

The absence of typical areas of focal midzonal necrosis and the presence of the rather distinctive "moth-eaten" areas of hepatic cell degeneration and necrosis are observations of considerable interest. It may be stated here that although the literature contains frequent statements to the effect that focal necroses do not occur in the cat following ligation of the common bile duct we have previously reported their consistent occurrence in this animal.[4] The absence of this lesion in the dehydrocholate-treated animals appears to contradict the hypothesis that its development is dependent solely on either the mechanical effects of increased intraductal pressure or on extravasation of bile into the parenchyma. The same statements may be made in this connection as were made in regard to the pathogenesis of the proliferation of bile ducts and associated connective tissue. The fact that the peculiar "moth-eaten" areas, which are not observed during simple stasis, appear during approximately the same period of stasis at which focal necroses would be expected to occur raises the possibility that these two processes are related pathogenetically. It is also possible that the "moth-eaten" areas of necrosis represent a stage in the development of focal necroses, the progression of the lesion being inhibited under the influence of dehydrocholate. However, morphologically, the two lesions are distinctive, differing from each other in appearance, number and distribution.

The persistence of stainable lipid in both hepatic and Kupffer cells with stasis of from eight to eighteen days' duration was in striking contrast to the almost invariable absence of this material in cats with uncomplicated stasis after the seventh day. It is also of interest to note the absence, as in simple stasis, of any consistent relationship between the distribution of stainable lipid and regressive changes; for example, stainable lipid could rarely be demonstrated in the "moth-eaten" areas of degeneration and necrosis. This observation suggests the possibility that the disappearance of stainable lipid during stasis is a result of diminished hepatic cell activity, since the administration of a supposedly stimulating substance (dehydrocholate) prevents this disappearance under otherwise identical experimental conditions.

Previous observations [4] of alterations in the concentration of bilirubin in the serum following ligation of the common bile duct in cats revealed that the bilirubinemia, although extremely variable, tended to

reach a maximum at about the sixth to the ninth day and subsequently diminished. The mechanism underlying this secondary decline was discussed, and the possibility was suggested that it is related to the diminution in hepatic cell activity incident to the disappearance of bile acids from the organism. This hypothesis is supported indirectly by the observations reported here. In animals receiving dehydrocholate the serum bilirubin does not exhibit this tendency toward a secondary fall.

The assumption seems logical that the administration of a substance such as sodium dehydrocholate, capable of stimulating hepatic cell activity, should exert some influence on the hepatic lesions associated with total bile stasis. It is interesting that with certain exceptions these lesions were not deleteriously influenced and indeed in some respects were apparently distinctly benefited by the administration of sodium dehydrocholate. Among the notable exceptions were the extensive areas of hyaline necrosis and the diffusely scattered "moth-eaten" areas of degeneration and necrosis. In cats in which lesions of the former type occurred the extent of hepatic damage was far greater than has been observed by us in any animal at comparable periods of simple stasis. Although focal necroses were not observed, the characteristic "moth-eaten" areas of degeneration and necrosis which occurred at comparable periods of stasis involved a greater proportion of the total hepatic parenchyma despite the fact that the individual lesions were much smaller. With these noteworthy exceptions the regressive changes characteristic of the liver in biliary stasis were much less prominent, the regeneration of hepatic cells was much more active, and the proliferation of small bile ducts and associated connective tissue was distinctly retarded. The latter phenomena are apparently dependent on the effect of the dehydrocholate, the mechanism of the action of which in this connection is not clear. These observations suggest that the regressive changes, the extensive proliferation of bile ducts and associated connective tissue and the relatively less active regeneration of hepatic cells during simple stasis may be results of the diminished stimulation of hepatic cell activity incident to the state of total stasis. It may be of interest to note, in addition, that no consistent relationship could be demonstrated between the changes in the liver and the extent or severity of the renal lesions previously described by us [2] following the administration of sodium dehydrocholate.

SUMMARY

Sodium dehydrocholate was administered intravenously, usually every twenty-four hours, to twenty-nine cats with total bile stasis of from two to eighteen days' duration and to a control group of twenty-two normal cats. Studies were made of the degree of bilirubinemia

and bromsulphalein retention and of the gross and microscopic changes in the liver and bile passages.

Certain distinct differences were noted in the condition of the liver in cats with uncomplicated stasis and in those with stasis which were receiving dehydrocholate. In the latter, focal midzonal necroses were not observed, but rather distinctive "moth-eaten" areas of degeneration and necrosis appeared at about the period of stasis during which the former would be expected to develop. In some cats there were extensive areas of hyaline necrosis which resulted in more widespread hepatic parenchymal damage than has been noted by us at comparable periods of uncomplicated stasis. With these exceptions the regressive lesions of stasis were less marked in animals receiving dehydrocholate.

Among the outstanding features in the livers of these animals were the relatively marked regeneration of hepatic cells and the distinct retardation of the proliferation of small bile ducts and associated connective tissue. Furthermore, the small proliferated ducts were almost invariably well luminized, in contrast to the solid, cordlike arrangement of the bile duct cells in simple stasis.

The persistence of stainable lipids in the hepatic and Kupffer cells throughout the experimental period was in contrast to the marked diminution in this material in the livers of animals after the seventh day of uncomplicated stasis.

Whereas in animals with simple stasis the concentration of bilirubin in the serum tends to diminish after six to nine days, this phenomenon was not observed in animals with stasis receiving dehydrocholate.

These observations suggest the possibility that certain of the regressive parenchymal lesions and the marked proliferation of small bile ducts and of associated connective tissue which occur during total bile stasis are not dependent entirely on the mechanical effects of the increased intraductal pressure or on the extravasation of bile into the adjacent parenchyma. The possibility is suggested that they are dependent in part on diminished stimulation of hepatic cell activity during stasis, due perhaps to diminished formation of bile acids.

Laboratory Methods and Technical Notes

A PERFORATED CUP FOR DEHYDRATION OF TISSUES WITH DIOXANE

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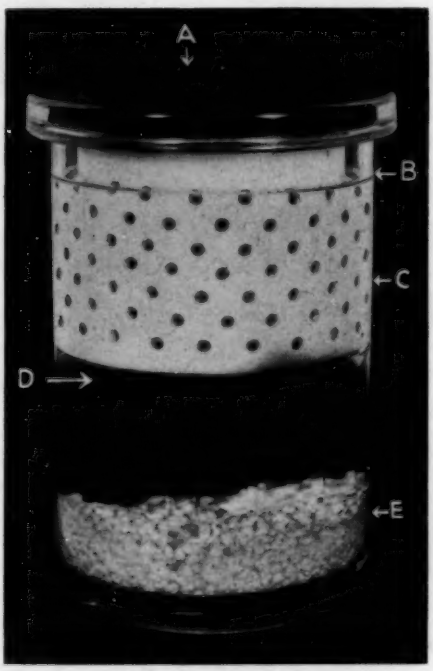
The present article contains a description of a perforated cup and of its use when dioxane is employed as a dehydrating and clearing agent in the preparation of sections of paraffin-embedded tissues for histo-



A perforated cup for the dehydration of tissues with dioxane: *A*, stender dish; *B*, level of dioxane; *C*, perforated cup; *D*, glass support; *E*, anhydrous calcium chloride.

logic study. Readers are referred to the articles of Groupner and Weissberger [1] and Mossman [2] for the details of the use of dioxane.

1. Groupner, Heinz, and Weissberger, Arnold: Zool. Anz. **96**:204, 1931.
2. Mossman, H. W.: Anat. Rec. (supp.) **58**:82, 1934.

Here, it suffices to say that the method depends on the miscibility of dioxane with water and paraffin, whereby dehydration and clearing may be accomplished in one step. The method is exceedingly economical when dioxane is employed with anhydrous calcium chloride to absorb the excess water from the tissues.

DESCRIPTION OF APPARATUS AND ITS USE

A Stender dish, 93 mm. in height and 62 mm. in diameter, having a tightly fitting ground glass lid, is employed to prevent evaporation and escape of fumes into the room, as dioxane is inflammable and somewhat anesthetic. A cylindric procelain cup, 5 cm. in diameter and 4 cm. high, with a flat bottom, serves to hold the tissues. The bottom and sides of the cup are perforated by numerous small openings, each about 2 mm. in diameter. The cup rests on a glass support consisting of a glass ring with four legs, which is 4 cm. high (figure).

The glass support is placed in the Stender dish, which is then filled to a depth of 1 cm. with anhydrous calcium chloride. The perforated cup containing the tissues is placed on the support, and the Stender dish is filled with dioxane to within 1 cm. of the top of the cup.[3]

With this apparatus, the tissues do not come in contact with the calcium chloride, and since it is composed of glass and porcelain there is no chemical reaction between the tissues, the dehydrating agent and the vessel. The position of the tissues in the center of the jar insures better dehydration as the water is heavier than dioxane and rapidly sinks to the bottom of the jar. The diffusion is rapid, and the water is quickly absorbed by the calcium chloride. Even though the fluid is used constantly, it will remain serviceable for a period of two or three weeks. After this length of time it can be filtered and, with more calcium chloride added, can be used again with practically no disadvantage.

3. The Arthur H. Thomas Company have fabricated the porcelain cup and stand according to our design.

REMOVAL OF THE SPINAL CORD THROUGH THE FORAMEN MAGNUM

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DETROIT

Present methods of removing the spinal cord at autopsy by an anterior [1] or posterior approach entail considerable time and effort. This fact has precluded routine examination of the spinal cord in many hospitals. It is our purpose to present an instrument and technic which greatly simplify the procedure and may be used whenever the autopsy includes examination of the cranial contents.

The instrument used (fig. 1) is a finely sharpened thin-bladed surgical steel knife affixed to a flexible wire. The knife, which is sharp on all sides, is 2 cm.

From the Department of Pathology, the Receiving Hospital.

1. Kernohan, J. W.: Am. J. Clin. Path. **3**:6, 1933.

in length and 1 cm. in width. The wire is 2 mm. in diameter and 60 cm. in length, having a handle which when maintained in the horizontal position insures the vertical position of the knife.

After removal of the brain, the brain stem is sectioned through the pons as close to the cerebellum as possible. The cranial nerves in this area are readily accessible and can be easily sectioned. With the instrument described, section of

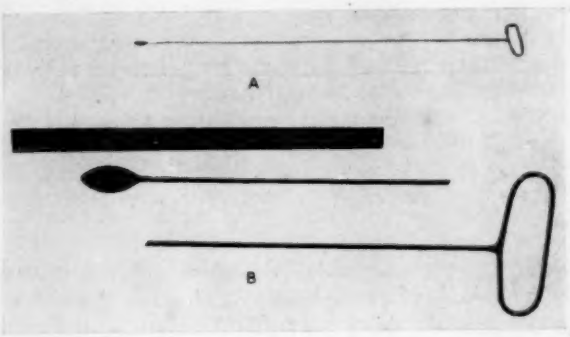

Fig. 1.—*A*, view of the entire instrument; *B*, view of the cutting head and the handle.

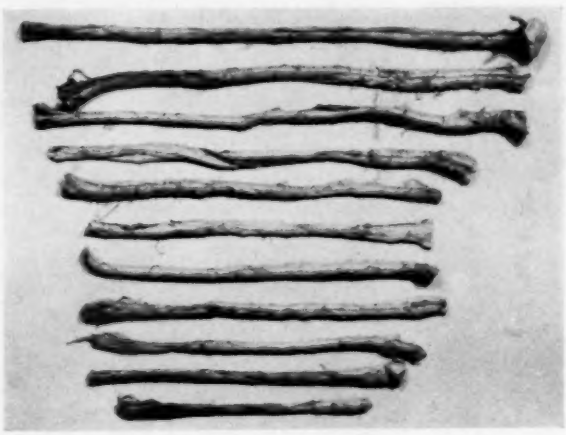

Fig. 2.—The first eleven cords removed by the method described.

the spinal nerves and the ligamentum denticulatum is next undertaken. The end of the knife is passed between the arachnoid and the dura along both lateral surfaces of the spinal cord. Tension may be put on the spinal cord by means of a finger forceps as the knife is progressively moved distally. Ready passage of the knife is assisted by keeping the head as nearly level with the thoracic vertebrae as possible by means of slight elevation of the head. A large sponge placed under the head is sufficient to obtain this.

When the spinal nerves have been sectioned, which requires but one insertion of the instrument along each lateral surface, with the handle held in the horizontal

position, the next step is incision of the ninth intervertebral disk and separation of the bodies of the ninth and tenth thoracic vertebrae. To facilitate this step a block 8 inches in height is placed under the midportion of the body and separation effected by means of a heavy, sharp knife. This V-shaped separation permits exposure of the spinal cord, which may be completely sectioned. It is important that a complete section be made. Failure to do this results in resistance to withdrawal through the foramen magnum, which is the next step.

It has been our experience that when resistance is met with in withdrawal of the spinal cord through the foramen magnum an alternative method may be used. The cartilaginous disks above and below the tenth dorsal vertebra are sectioned by means of a heavy, flat-bladed knife. By means of a chisel the body of this vertebra is split in a longitudinal plane, thus exposing the dura of the spinal cord. The dura and spinal cord are then sectioned, and by means of a Kelly forceps the spinal cord may be withdrawn distally. This is effective when unsectioned spinal nerves at the distal portion of the cord offer too great resistance to tension at the foramen magnum.

Thus, within ten minutes, with little effort and with no trauma to the cord proper, the spinal cord may be removed either through the foramen magnum or through the space formed by removing one vertebra body (fig. 2).

In those cases of pathologic changes of the spinal cord or associated conditions in which the dura and surrounding structures must be examined this method is not desirable, and dissection is preferable. For routine examination it is our experience that this method is of value, and we recommend it to the attention of those in large pathologic services.

The instrument may be obtained from the J. F. Hartz Surgical Supply Company, Detroit.

Case Reports

ARTERIOLAR DISEASE OF THE HEART

Associated with Severe Myomalacia; Coronary Arteries Intact

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The causal relationship of myomalacia and disease of the coronary arteries is generally recognized. The more slowly developing diffuse fibrosis of the myocardium in most cases is related similarly to disturbances of coronary blood flow. The anatomic changes in the coronary arteries are not always proportional, however, to the changes in the myocardium (V. Levine,[1] Fitzhugh,[2] Brown,[3] Lisa and Ring [4] and S. A. Levine [5]). The importance of various other factors, such as collateral circulation, anemia, increased need of oxygen during exertion and wasteful use of oxygen by the failing heart is fairly well established. Changes in the myocardial arterioles, however, and their effect on myocardial nutrition have received little attention. We have observed a case illustrative of this type of disturbance.

REPORT OF A CASE

A 46 year old Jewish housewife was admitted to the service of Dr. I. W. Held in most severe circulatory failure. No reliable previous history was obtained. She had suffered from an episode of acute circulatory collapse six weeks previously. This was followed by persistent shortness of breath and swelling of the legs. Several attacks were experienced during the subsequent weeks, the last one occurring four days before admission to the hospital. No definite history of pain in the chest could be elicited. The extreme degree of circulatory failure did not lessen, and death occurred forty-eight hours after admission. Unfortunately, no electrocardiographic studies were available.

Most of the organs of the chest and abdomen were available for postmortem examination. The lungs showed nothing unusual aside from slight emphysema and a scar in the left apex; the bronchi and pulmonary blood vessels were intact.

The heart was slightly larger than normal, the auricles being moderately distended; there was no valvular lesion. The myocardium was firm, somewhat glassy, pale flesh-colored, with numerous irregular yellow areas. The coronary arteries to the naked eye appeared perfectly intact.

The spleen was small, its markings normal. The left kidney had a few irregular cyanotic depressions but otherwise was not remarkable. The right kidney was considerably enlarged, and the surrounding fat tissue was adherent to it. A large

From the Pathological Department, Beth Israel Hospital.

1. Levine, V.: Arch. Path. **18**:331, 1934.
2. Fitzhugh, G.: New England J. Med. **203**:201, 1930.
3. Brown, M. R.: Am. J. M. Sc. **184**:707, 1932.
4. Lisa, J. R., and Ring, A.: Arch. Int. Med. **50**:131, 1932.
5. Levine, S. A.: Medicine **8**:245, 1929.

concretion distending the upper part of the ureter had caused hydronephrosis. The liver was small, with some fatty change and slight stasis. The other abdominal organs were intact.

Many pieces from different portions of the heart muscle were examined. The slides were stained with hematoxylin and eosin, the Van Gieson mixture and Weigert's stain for elastic tissue. The results of the examination were as follows: The large coronary arteries were intact. Most medium-sized branches were also intact or nearly so. Very many small branches had the appearance of severe arteriolosclerosis. Some medium-sized arteries had large homogeneous hyaline areas in the outer layers of the muscle coat, while the inner layers were normal. In some of the sections one gained the impression that either the subepicardial or subendocardial layers harbored more diseased vessels than other layers. Taking the average of a large number of slides, one found no preponderance of the arterial lesions in any portion of the heart. On the whole, the vessels with a diameter below 0.1 mm. were diseased, while the larger ones for the most part were intact. (Measurements were taken between opposite points of the internal elastic lamella.)

In sections stained with hematoxylin and eosin these arteriolar lesions did not differ from ordinary hyalinization as found in various organs of arteriosclerotic patients and in the spleens of most adults. There was, however, an unusual preponderance of hyalinization in the muscle coat. The intima in general was intact. The internal elastic lamella was situated directly at the lumen with only the more or less well preserved endothelium internal to it. Only occasionally did one see a vessel with some intimal overgrowth. The homogeneous masses in the arterial wall were for the most part situated outside the internal elastic lamella. A few vessels showed the internal elastica within the homogeneous mass, in which the inner edge of the media could not be recognized.

This description of the arteriolar lesion applies to vessels which were not surrounded by myomalacia. There was, however, astonishingly little difference between arterioles in the normal myocardium and in the severely diseased areas.

The other organs did not show arteriolar lesions except for the kidneys, in which occasional vessels were involved as in any middle-aged or older person. No material from the pancreas, adrenal glands or brain was available for examination. There were no other important microscopic observations in any organ.

OBSERVATIONS ON SMALL VESSELS IN SEVENTY-ONE HEARTS

On the basis of these findings, a study was made of the coronary artery system in seventy-one human hearts, with special attention being devoted to the small branches. Tables 1 and 2 indicate the types of material. Sections were taken from both circumflex arteries, the anterior and posterior descending branches in the middle of the ventricle, the left ventricle 2 cm. above the apex anteriorly, the anterior papillary muscle longitudinally, including its base, and the ventricular septum. The slides were stained as described. The condition of the vessels of various calibers was noted and recorded.

In the group of hearts with involvement of the main coronary arteries, ranging from a mild degree of sclerosis to complete closure of one or more large vessels, the vessels of smaller caliber (small arteries, arterioles and capillaries) were strikingly unaffected. The involvement of the large arteries was not associated in any case with any corresponding change in a significant number of the smaller vessels in the heart. The myocardial damage noted varied from large zones

of recent infarction and scars of previous necrosis to the diffuse change of myocardial fibrosis. The small vessels in these large or small affected areas showed no essential changes. Occasionally the media became intensely red in the eosin stain. Marked sclerosis of an occasional isolated arteriole was seen, most often in the epicardium, but this did not appear of significance in nutrition; this finding was most frequent in the older age group. No heart was found with arteriolar changes even approaching in extent or degree those of our original case.

TABLE 1.—*Cardiac Material Studied*

	Hearts
1. Rheumatic lesions	17
Acute rheumatic carditis	2
Acute and chronic rheumatic carditis	15
Acute and chronic rheumatic carditis with superimposed bacterial endocarditis	6
2. Sclerosis and thrombosis of the coronary artery	14
3. General arteriosclerosis and arteriolosclerosis	9
4. Myocarditis	2
Acute septic myocarditis	1
Chronic myocarditis	1
5. General amyloidosis	1
6. Glomerulonephritis	2
Acute	1
Chronic	1
7. Miscellaneous nonvascular diseases	26
Total	71

TABLE 2.—*Distribution According to Age*

Decade	Hearts
1st	5
2d	1
3d	6
4th	8
5th	14
6th	12
7th	18
8th	6
9th	1
Total (37 from males, 34 from females)	71

In the rheumatic group one heart only showed vessel changes as described for rheumatic fever. These in no way resembled the vessel changes described in our case. The remaining hearts showed no disease of vessels.

In the miscellaneous group no significant lesions of small vessels were seen.

COMMENT

Many histologic studies of normal and abnormal coronary arteries confirm the observation that there is no direct relationship between structural changes in the large and small vessels. Wolkoff [6] noted the infrequency

6. Wolkoff, K.: Beitr. z. path. Anat. u. z. allg. Path. **82**:555, 1929; Virchows Arch. f. path. Anat. **241**:42, 1923.

of involvement of small vessels in the "physiological intimal thickening" of aging. Kusnetzowsky,[7] too, found the small branches relatively unchanged and noted thickening of the intima only in advanced age. Gross, Epstein and Kugel [8] found the fibro-elastic metamorphosis of aging in the myocardial arteries but to a lesser degree than in larger coronary vessels. The arteriolar changes were still less marked.

True arteriosclerotic changes also appear first in the main branches of the coronary arteries and are most marked here. The process gradually extends distally and finally may affect small branches (Wolkoff,[6] Schultz). Jores [9] and Thorel noted that small branches of the coronary arteries were free from intimal thickening even when the large branches showed severe arteriosclerosis and calcification. Bork [10] and Kusnetzowsky [7] maintained, too, that the subepicardial and intramuscular twigs are affected far less frequently than the large branches, and that such involvement is mild despite most severe involvement of the large stems. The changes in the small vessels, according to several authors, occur most often in the substance of the papillary muscles of the left ventricle. Changes are more marked in the small branches under the epicardium than in those of the myocardium.

We did not find in the literature any reference to a case like ours, one, namely, of severe widespread myomalacia with markedly diseased myocardial arterioles, the large coronary vessels being intact.

Saphir [11] found no relation between the gross lesion in the larger branches and the histologic changes in the arterioles. The latter often showed little change in the presence of marked arteriosclerosis. Fitzhugh [2] commented on the lack of correlation between the amounts of sclerotic thickening in the coronary arteries and their small branches. He, however, found a relatively high degree (17.7 per cent) of involvement of the small vessels. Fitzhugh [2] did not state what his criteria for classification were and provided no photographs.

Levine,[1] studying the myocardial changes in hypertension in a series of twenty-seven hearts, found six hearts with marked sclerosis in the arterioles in one or more sections and thirteen with slight sclerosis of insignificant degree. In only one heart did marked sclerosis of the coronary arteries coincide with arteriolosclerosis of high degree. Fishberg [12] found involvement of the arterioles of the myocardium in only 3 per cent of cases of essential hypertension.

Sutton and Brandes [13] were impressed with the frequency of sclerosis about the ostia of the smaller coronary branches, even when the large artery showed no evidence of arteriosclerosis. (We have not been able to corroborate this observation in our material.) Kusnetzowsky [7] without giving details refers to an occasional finding of more marked involvement of small vessels.

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7. Kusnetzowsky, N. J.: Virchows Arch. f. path. Anat. **245**:55, 1923.
8. Gross, L.; Epstein, E. Z., and Kugel, M.: Am. J. Path. **10**:253, 1934.
9. Jores, L.: Virchows Arch. f. path. Anat. **178**:367, 1904.
10. Bork, K.: Virchows Arch. f. path. Anat. **262**:646, 1926.
11. Saphir, O., and others: Am. Heart J. **10**:762, 1935.
12. Fishberg, A. M.: Hypertension and Nephritis, ed. 2, Philadelphia, Lea & Febiger, 1931, p. 180.
13. Sutton, D. C., and Brandes, W. W.: J. Lab. & Clin. Med. **16**:1185, 1931.

We cannot explain this restriction of disease to the small vessels of the heart in our case, and we feel unable to classify it. The hyalinization of the arteriolar wall is the same as customarily found in arteriolosclerosis although, as mentioned, the prevalence of the lesion in the media is somewhat unusual. We must not forget, however, that in the spleen this localization of the hyaline masses is the usual one. If we assume (with Hueck) that imbibition of fluid or semifluid material represents this process of hyalinization we are not astonished to find variation in the localization within the wall of the vessel. Even the unusual distribution presented by some vessels in this heart, namely, hyalinization of outer layers of media with relatively intact inner layers, sometimes occurs in so-called ordinary cases of arteriolosclerosis. On purely morphologic grounds we thus might designate our case as one of arteriolosclerosis of the heart. The absence of corresponding renal lesions, however, points to a disease process of different character. Whether or not our patient has had hypertension is unknown.

SUMMARY

In a middle-aged woman who died of circulatory failure widespread myomalacia of the heart was found. The large branches of the coronary arteries were perfectly intact. Many small branches were severely hyalinized. The arterioles in the kidneys, intestine, ovary, uterus and lung showed nothing unusual. Extensive examination of seventy-one hearts failed to show any important lesion of the small vessels. The case cannot be classified.

General Review

"ALKALI DISEASE"—SELENIUM POISONING

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DEFINITION

Selenium poisoning, formerly called "alkali disease," is a newly recognized, often fatal poisoning among live stock of the North Central Great Plains region of the United States. It is due to the ingestion of plant materials which absorb from the soil the rare element selenium in toxic quantities. Its etiologic features have been thoroughly studied. The malady is characterized by distinct symptoms and lesions. This disease is not related to the "alkali disease" of the Pecos Valley or to the poisoning caused by the ingestion of highly alkaline waters.

HISTORY

In 1857 Surgeon T. C. Madison described a peculiar disease at Fort Randall which was located on the Missouri river in the territory of Nebraska (now Gregory County, South Dakota). The report is as follows:

> A very fatal disease manifested itself among the dragoon horses which is supposed not to have been described in works on veterinary surgery. Four companies of the second dragoons arrived at this post about the 10th of August, 1856, one squadron from Fort Lookout and one from the Big Sioux river, the latter accompanied by a number of new or remount horses. The four companies encamped on the east or lower side of the dry ravine separating the dragoon and the infantry camps. About the 20th of August the disease commenced simultaneously in all four companies and many horses died, not, however, until the lapse of weeks and months. The following symptoms were observed: first, among the remount horses . . . there was a sort of catarrh, or distemper with running at the nose and among all the horses a swelling of the skin of the throat and jaw; also inflammation, swelling and suppuration of the sheath, tenderness and inflammation of the feet, followed by suppuration at the joint where the hoof joins the skin, the hoof in instances detaching itself and a new one forming in its place. These were also accompanied by loss of the manes and tails; the appetite was uniformly good; but, from the extreme tenderness of the feet, they were unable to move about in search of food, and it appears that at that time they were entirely dependent upon grazing, there being no forage at the post for issue. Sorrel horses appeared to suffer most but no color escaped. The private horses of the officers shared the fate of the public animals. A few mules and Indian ponies were similarly affected. The acclimated suffered equally with the unac-

From the John McCormick Institute for Infectious Diseases.

climated. No treatment was effectual or afforded permanent relief. Bleeding in the feet was tried but its effect was merely temporary. Every case of the disease originated on the lower side of the dry ravine above alluded to. After forage was provided for the horses no new cases occurred, and hence, it is fair to infer that a liberal allowance of forage in the beginning might have rendered the disease much less fatal, or have prevented it. Whether the disease was caused by eating any poisonous herbs, or propagated in any manner by contagion could not be satisfactorily determined, the origin and progress of the malady being something entirely new, even to the Indians and half-breeds of the country. One postmortem was made of a horse that died late in the winter but developed nothing important; one lung was much diseased, about one-third of the stomach denuded of its inner lining and contained botts and the large intestines inflamed. It is well to remark . . . when these noble animals became seriously sick they almost invariably died.

In the autumn and winter of 1890-1891, according to Mayo, severe losses of horses from a new and strange disease called "blind staggers," "mad staggers," or "staggers" were reported. It was observed that wherever in Kansas corn was raised and fed the disease was common. Within a radius of 20 miles from Manhattan, Kansas, there were fifty deaths among the animals. The disease was not confined to any locality, and it occurred with equal measure in the hills and in the river valleys, as well as in well cleaned stables, and was prevalent among horses pastured in fields. Nothing could be found wrong with the drinking water. The symptoms of horses in this region were anorexia and refusal to drink, dulness, drooping of the head and ears and partial or complete blindness. They lost consciousness, became delirious, fell into a coma and died. Some lost control of their hind parts, others pressed their heads against a post or wall, and still others were thrown into a frenzy by excitement. The temperature varied between 100 and 101 F., the mucous membranes were injected, the pulse rate ranged between 40 and 100, the urine was scanty and high colored, often containing blood and pus, and the bowels were constipated. The postmortem examinations by Mayo revealed much congestion of the liver and hyperemia of the gastro-intestinal tract and of the brain.

In 1893 the Wyoming Agricultural Experiment Station observed this peculiar disease among horses in Carbon County, Wyoming, and in 1897 and 1904 Peters encountered it among domestic animals in Boyd County, Nebraska. The symptoms were loss of flesh, partial or complete depilation of manes and tails, elongation and cracking of hoofs and erosions of the long bones. Hogs lost their bristles, and their hoofs began to slough. They became gaunt and many died. Chickens showed great loss of feathers, and the eggs were infertile. Peters and Hunter stated that farmers had reported this disease since 1891. One farmer lost three hundred hogs and three horses, and one hundred of his cattle were ill. He was unable to raise chickens because the eggs did

not hatch. Many other farmers reported similar experiences and noted that a change of food controlled the disease. In 1922 Lipp described the disease in certain parts of South Dakota along the Missouri river.

ETIOLOGY

The causative agent remained obscure until recently. Blame had been placed on impaction of the stomach with coarse foods, poisoning of the vegetation by spiders, poisonous gases produced in the soil or vegetation, intensive sunlight, deficiency of the glands of internal secretion and ergotism. Mayo found in every case a history of eating corn infected with the mold Aspergillus glaucus, and Peters, Hunter and Sheldon isolated from corn the fungus Fusarium and confirmed their views in animal experiments with each of these agents. The studies of the Wyoming Agricultural Station from 1921 to 1930 ruled out bacteriologic and contagious factors. Most farmers in the affected districts condemned the alkali in the water or soil and thus named the disease "alkali disease." Roderick suggested the importance of rickets and a deficiency of phosphorus.

In 1928 Franke of the South Dakota State College of Agriculture learned that animals consuming vegetation growing on particular areas acquired "alkali disease." He found that grain which had been grown on "alkalied" farms was toxic to white rats, and he attributed the injurious effects to a definite toxic constituent or group of constituents. In 1931 Knight of the Bureau of Chemistry and Soils, United States Department of Agriculture, suggested that traces of selenium absorbed by plants from the soil might be responsible. Analysis of a sample of toxic wheat by Robinson revealed from 10 to 12 parts of selenium per million. These important discoveries stimulated investigation, conducted for the most part by the Bureaus of Chemistry and Soils, Plant Industry and Home Economics of the United States Department of Agriculture, Washington, and the agricultural experiment stations of South Dakota and Wyoming and others. The evidence obtained indicates that the "alkali disease" of live stock of the North Central Great Plains may be due to traces of selenium in the indigenous grains and forage.

Of the states of the North Central Great Plains, "alkali disease" has been reported with greatest frequency in South Dakota and Wyoming; it is thought that North Dakota, Kansas, Montana, Nebraska, Colorado and New Mexico are involved. The incidence in these respective states is unknown. The confusion of rayless golden-rod poisoning, epizootic, senecio poisoning or walking disease with "alkali disease" diminishes the value of the few data available. Byers has reported selenium in soils and vegetation in the country bordering on South

Dakota, Kansas and Colorado. In their survey in 1931 through the central and southwestern parts of South Dakota, parts of northern Nebraska and the eastern edge of Wyoming Franke, Rice, Johnson and Schoening found the malady locally in horses, cattle, swine, poultry and mules (very few sheep were found in the affected territory) and submitted the following report:

> Losses vary with the conditions. Where the disease is severe considerable live stock may die or have to be killed and eggs cannot be used for hatching purposes. In such localities, the raising of hogs or poultry, or both, and even cattle, has been discontinued, and tractor-farming of small grain for the market is the sole substitute. Such grain, known to have been produced on affected soil, brings only minimum prices. In two specific cases, noted in 1931, the socalled "alkalied" grain brought only half the price of good grain from unaffected farms. Likewise, affected animals usually bring only minimum prices when marketed. Horses that are so severely "alkalied" as to lose their hoofs cannot be worked for months, during which time they require special care. In some cases they become repeatedly "alkalied," so that they cannot be worked for a year or more. Owners of some badly affected farms have abandoned them, chiefly on account of this trouble, not being able to finance, lease, or sell them. In many cases new renters of affected farms, unfamiliar with conditions, have had heavy losses within a year after moving to such farms.

Having demonstrated the presence of selenium in vegetation growing on "alkalied" soils, in the soils on which crops are grown and in the shales from which these soils are derived, the Bureau of Chemistry and Soils regards the condition with concern. Grain and other plant materials occupying an important place in the diet of man and animals are leading crops in the affected areas and have been found to contain selenium. Because of the presence of selenium in wheat not alone from the North Central Great Plains but also from the vast wheat-growing fields of Canada, Australia, Argentina and other countries the problem may be of wide importance.

Byers gives the parts of selenium per million parts of foods obtained in an "alkalied" area as follows: milk, from 0.3 to 9.6; egg (white, yolk, shell), from 1 to 10; corn, 3; wheat, 5; rye, 5; turnip leaves, 25; string beans, 2; lettuce, 7; cabbage, 150. Selenium has been found by many other investigators in toxic corn, wheat and barley. Data like these indicate the seriousness of the problem.

"Alkali disease" is essentially one of arid and semiarid, nonarable districts, where selenium is found in greatest amounts. Some of these areas are used for agricultural purposes, mostly grazing. In Wyoming, according to Beath, the disease occurs primarily on the range and not on the farm. There is geographic as well as seasonal variation in the toxicity of the vegetation. The total selenium content of the soil does not indicate the degree of toxicity of either the soil or the plants growing on that soil. In humid areas some selenium has been found in soil,

but the vegetation is not usually toxic. The toxicity of vegetation depends on its selenium content, and the absorptive power of plant life for selenium depends on the degree of rainfall, leeching, weathering, composition of the soil, the geologic outcrop on which the plant material grows, the species of plant and the amount of available sulfur or sulfate in the soil. Any soil containing more than 0.5 parts of selenium and any vegetation containing 5 parts of selenium per million is potentially dangerous (Byers).

Selenium occurs in the cretaceous and eocene formations in the North Central Great Plains. During these periods sediments resulting from erosion were layered in the Rocky Mountain area in series or bands deposited at different intervals of time, and a few of the series containing selenium in high content are the Niobrara, Steele, Pierre and Wasatch. Especially important is the Pierre formation which has been found to contain from traces to 150 parts of selenium per million (Byers). It extends over a vast territory including North Dakota, South Dakota, Nebraska, Montana, Wyoming, Colorado, New Mexico and perhaps other territory. Outbreaks of the malady have been reported in regions where the soils are derived from one of these formations. All soils derived from pyritiferous materials contain selenium unless leeching has occurred. Dangerous amounts of selenium are found in heavy clays; sandy soils have shown only traces and alluvial soils little or none. Selenium does not occur to an appreciable degree in water in these areas.

The element selenium, atomic weight 78.9, was discovered by Berzelius in 1817 and named in tribute to Selene, Greek goddess of the moon. Its intimate association with sulfur has long been recognized, and it possesses many of the physical and chemical properties of sulfur as well as of tellurium and arsenic. The common forms are hydrogen selenide (H_2Se), sodium selenide (NaSe), potassium selenide (K_2Se), selenium dioxide (SeO_2), selenious acid (H_2SeO_3), sodium selenite (Na_2SeO_3), selenic acid (H_2SeO_4) and sodium selenate (Na_2SeO_4).

TOXICITY OF SELENIUM

The toxicity of selenium varies with its ionic state (Gmelin; Czapek and Weil; Stoklasa; Joachimoglu and Hirose; Woodruff and Gies; Franke and Potter; Hurd-Karrer and others). Metallic selenium is not toxic or very little so to bacteria, plants and animals. Selenium salts are very toxic, however. The sequence of toxicity for animals arranged from greatest to least, according to Franke and Moxon is: sodium selenite, sodium selenide and sodium selenate. The order of toxicity for plants is: selenious acid, selenic acid, sodium selenite, sodium selenate and potassium selenocyanide (Levine). In contrast

to most other authors, Hurd-Karrer observed that sodium selenate is more toxic (to wheat) than sodium selenite. Czapek and Weil found that 0.5 mg. of sodium selenite caused death in the frog, and 4 mg. per kilogram killed the rabbit, cat and dog in a few hours, and to produce the same effect five times that dose of sodium selenate was required. A moderate-sized rabbit died after the administration of 0.5 cc. of a 2 per cent solution of sodium selenite, and a moderate-sized cat, after 1 cc. of a 2 per cent solution, Jones reported. Knight observed that sodium selenate is lethal in doses of 3 mg. and organic selenium in quantities as low as 2 mg. per pound of animal. For the rat Franke and Moxon have found the minimum lethal dose of sodium selenate by intraperitoneal injection to be from 5.25 to 5.75 mg. per kilogram of body weight and that of sodium selenite from 3.25 to 3.5 mg.

A dose of from 3 to 4 parts of selenium per million in either food or feed is not dangerous, but 15 parts per million is dangerous and produces selenium poisoning. Byers said that the toxicity of the vegetation is produced by 8.5 parts of selenium per million. It is assumed that 4 parts per million is the limit of tolerance. The lethal dose of selenium as it occurs in green vegetation is about from 2 to 4 mg. per pound of body weight for the larger animals.

Arsenic and selenium have similar toxic effects. Living cells have the ability to reduce the toxic salt to the inert substance, a fact appreciated by the earliest investigators. It is thought that the selenate, which is comparatively stable and less toxic, is changed by the body tissues to selenite, the extremely unstable and very toxic form, and that this is easily reduced to metallic selenium.

CHEMICAL FORM OF TOXICANT

The chemical combination in which the toxicant exists in the plant and the soil is not known thoroughly. Knight observed that selenium compounds may be extracted with hot water from toxic vegetation, but most of the toxicant is combined with the protein fraction and insoluble in water. Beath, Eppson and Gilbert reported that alfalfa does not in the natural state absorb selenium but that when a water extract of selenium-containing Astragalus bisulcatus is added to soil in which alfalfa is growing the alfalfa when fed to animals is toxic. The experiments of Franke illustrate that most of the toxicant in wheat and corn is present in the protein fraction, which when fed to rats produced the typical pathologic condition with destructive changes in the liver, spleen and reproductive organs. Selenium exists in an organic combination and does not occur to any appreciable amount as metallic selenium or as inorganic selenium salts. Painter and Franke found mercuric chloride to be the best precipitant for selenium compounds.

They believe that selenium exists in a compound similar to cystine or cysteine. E. M. Nelson has showed that the toxicant of seleniferous wheat is concentrated in wheat gluten (Byers and Knight). It is probable that selenium occurs in both the organic and the inorganic form in toxic soil, and it has been obtained by extraction of soil with both water and ammonium hydroxide.

SYMPTOMS

Natural Selenium Poisoning.—The animals affected are usually cattle, horses, swine, sheep and chickens, rarely mules. The young are more prone to the poisoning than the old, and grazing animals are the usual subjects. In certain localities the symptoms are acute, in others chronic. Animals in an affected area may be subnormal without being noticed. Diseased animals appear prematurely old. The ranchers entitle the acute disease "blind staggers," but some of the animals affected with it are neither blind nor do they stagger. Symptoms develop within a variable period of time depending on the degree of intoxication and the type of vegetation ingested, usually in from a few hours to several days, according to the observations of Beath, Draize and Gilbert. The animal straying from the herd shows signs of impairment of vision, is unable to judge the height or distance of objects in its path, and eventually displays pronounced blindness. The eyelids are edematous, and lacrimation is profuse. The aqueous becomes swollen and turbid, and the cornea often shows a hemorrhagic opaque white mass protruding beyond the curvature of the eyeball, making the limbus of the cornea indistinguishable. The coat is roughened and off color. The ends of the long bones are eroded in two thirds of the affected animals, resulting in great difficulty in walking. The animals refuse to eat and often they eat a plant such as salt bush (Atriplex Nutalli). The appetite becomes so depraved that the animal chews fence rails, pieces of bone and nails. The sheep are affected in a similar manner, and many of them have weak lambs, which may be so malnourished and exhausted that they cannot stand and may die of starvation; they eat foreign material, and the intestinal tract may contain wool, sticks and metal pieces in large quantities. Severely affected cattle wander aimlessly about in circles. There is some evidence that they are suffering from severe abdominal pain. They grate their teeth, drool and grunt; paralysis sets in and then death. Many of them show at repeated intervals quick starts. Respiratory failure is the cause of death.

"Alkali disease," the chronic form, has been carefully described by a number of observers (Madison; Peters; Lipp; Beath, Draize and Gilbert; Franke, Rice, Johnson and Schoening, and others). Like "blind-staggers," "alkali disease" interferes with growth, normal devel-

opment and reproduction. The condition may be mild or severe. Stunting of growth may be marked, especially in the young animals. In horses, cattle, sheep and swine the horn of the hoofs is elongated, assuming bizarre shapes. One digit may grow over its adjacent fellow and may grow to two or three times the usual length. Often horizontal or vertical cracks occur in the digits and may reach the weight-bearing part of the foot, resulting in soreness and limp. Involvement of the forehoofs leads the animal to graze in a kneeling position, and sometimes the soreness of the feet forbids all activity and terminates in death from thirst and starvation. Often the old hoof sloughs, and lameness lasts for months. The sick animal walks with a peculiar gait as though trying to clear a low fence in its path or a high object. The hair is lost from the mane and tail of the horse, switch of the cow and body of the hog. No ocular symptoms are present. The course is similar to that of "blind-staggers," with death from exhaustion and toxemia. Draize and Beath have visited ranches where the affected cattle show marked stunting in growth and the offspring degeneracy. The importance of this disease in the causation of sterility is not known, but some think the reproductive power is impaired. Removal of very severely affected animals from "alkalied" areas to pastures containing normal forage may bring about recovery, but many stockmen believe the damage is permanent. Affected young pigs placed under these circumstances gain little or no weight despite nutritious feeding. Beath has recognized the possibility of delayed action in selenium poisoning; often symptoms may be delayed for many months after the animals have grazed on toxic vegetation, but suddenly these animals become acutely ill and die in from one to six days after they suddenly present hematuria and loss of weight.

Franke and Tully reported that for a number of years farmers in South Dakota observed that chicks did not hatch satisfactorily either under natural or under artificial conditions, that the down on chicks which did successfully hatch had a greasy appearance and failed to become fluffy, and that when higher hatchability was obtained mortality became higher. Eggs from an affected farm were incubated, and most of the embryos died within eighteen days. Many of these were monsters (terata) showing the following malformations: edemic swelling over the back of the head, an incompletely absorbed yolk sac abnormally located, crooked spindly legs, joined toes, a rudimentary upper beak, absence of the upper beak, absence of a wing, stubbed wings, stubbed legs, absence of an eye, absence of both eyes, cyclopean eye, rudimentary eyes, one toe and foot, and other abnormalities. The down was coarse and greasy as reported by the farmers. Approximately 75 per cent of the eggs failing to hatch on the twenty-first day contained deformed embryos. The hatchability was 4 per cent of the fertile eggs in one lot and 12 per cent in another.

Experimental Selenium Poisoning.—The symptoms produced experimentally with toxic vegetation and with selenium compounds are very similar to those encountered in natural selenium poisoning (Franke; Franke and Potter; Schneider; Tully and Franke; Munsell, DeVaney and Kennedy, and others). Loss of weight and failure to gain weight are prominent in white rats and chicks. The rats take a hunched position and prefer to be still; soon they show subcutaneous hemorrhages, hemorrhages about the joints and occasional paralysis of the hind legs. Cachexia develops in those with chronic poisoning, and death occurs early in those with acute poisoning. Fertility is markedly diminished. Chicks show ruffled feathers and nervousness, and the laying of eggs is delayed and reduced. Franke, Moxon, Poley and Tully produced monsters by injecting (before incubation) sodium selenite and sodium selenate into the air cells of hens' fertile eggs. The abnormalities encountered were found to be similar to those occurring on "alkalied" farms due to toxic grain.

Animals poisoned with toxic vegetation and inorganic selenium show anemia of varying grades. The moribund animals may have a red blood cell count as low as 450,000 per cubic millimeter (Franke and Potter) and a hemoglobin content of 2 Gm. per hundred cubic centimeters of blood (Schneider). The erythrocytes display marked achromia, anisocytosis and polychromatophilia. The acutely poisoned animals manifest a tendency toward leukopenia, and the differential leukocyte count reveals an alteration in the normal ratio of the various white cells.

Animals given injections of selenium show complete absence of free hydrochloric acid from the stomach (Levine; Jones), partial or complete disappearance of fat from the stool (Jones), increase in urea and phosphate (Duhamel and Rebiere) and rise in total nitrogen and urea and fall in creatinine (Cathcart and Orr) in the urine. Selenium appears in the urine and blood of animals fed either organic or inorganic compounds of selenium (Dudley; Dudley and Byers). It is also present in the milk of lactating animals that are affected.

Selenium Poisoning in Man.—Little is known of selenium poisoning in man. Tunnicliffe and Rosenheim described poisoning from accidental ingestion of beer containing selenic acid. The symptoms were paresthesias, erythromelalgia and melanosis, which were later attributed to arsenic. Hamilton reported an outbreak in a copper plant where several men complained of bronchial irritation, head cold, garlic odor to the breath, abdominal and lumbar pain, vomiting, night sweats, sore throat and fever. Dudley demonstrated in the urine of male employees of a copper refinery selenium in concentrations varying from traces to 6.9 parts per hundred million. Some of these men showed pallor, coating

of the tongue, gastro-intestinal disturbances, nervousness and pronounced garlic odor to the breath. The occurrence of selenium poisoning in many varieties of animals within the affected territory suggests its possibility in man, a subject which is now under investigation. No deaths from selenium poisoning have been reported in the toxic areas.

PATHOLOGIC ANATOMY

The essential lesions of "blind staggers" and "alkali disease" concern the liver and weight-bearing bones, though other structures are affected to lesser degrees (Franke; Draize and Beath; Peters and Hunter; Mayo, and others). Autopsies on one hundred "alkalied" cattle and sheep by Draize and Beath revealed these involvements. The gastro-intestinal tract is in a state of atony, the rumen is dilated, and there are inflammation, hemorrhages and degenerative changes involving the epithelium and substrata of the longitudinal folds or leaves of the omasum. The omasum and jejunum are hyperemic and in severe poisoning show ulceration and petechial hemorrhages. The peritoneum shows injection, and ascites is frequent. The gallbladder is usually enlarged, and the mucosa shows mucoid degeneration and hyperemia. The liver in "blind staggers" is acutely congested with many areas of focal necrosis surrounded by zones of hemorrhage, and the surface is scarred; in "alkali disease" cirrhosis is extensive, with diffuse scarring and marked atrophy. In the early stages there is widening of blood-filled sinusoids, especially in the region of the central veins, and these undergo necrosis with new formation of hepatic cells. In addition to these changes, in the later stages there is abundant connective tissue proliferation about the portal triads. Atrophy of the hepatic cells about the central veins occurs. The bile ducts show regenerated epithelium. Considerable perivascular leukocytic infiltration is present. The heart is flabby ("dish rag") and contains subendocardial, subepicardial and myocardial hemorrhages. The acute and severe forms reveal infiltration of the myocardium by lymphocytes, and the muscle elements are occasionally atrophied; the chronic forms show marked atrophy of the individual muscle fibers. Hydropericardium is frequent, with the lungs congested and the alveolar walls thickened. The renal medulla shows injection; mild glomerulitis is present; the epithelium of the tubules, especially of the convoluted ones, is degenerated, and severe intertubular hemorrhage is present. Stones may be found in the renal pelvis. The spleen, firm and atrophied in the chronic condition congested in the acute, shows heavy deposits of hemosiderin in the pulp. The lymph glands are reddened and edematous. The ends of the long bones show erosions. In "alkali disease" the hoofs are typically abnormal. They are elongated and oddly shaped; one digit may grow over the one adjacent to it or it may grow to two or three times the

length of its fellow. Often horizontal or vertical cracks occur in the digits and may reach the weight-bearing parts of the foot, accounting for the difficulty in walking. The bone marrow is gelatinous.

Franke and his associates observed in a horse and cow suffering for two years from "alkali disease" erosions of the articular surfaces of many of the joints and edema of the joint tissues as well as gelatinous degeneration of the bone marrow.

The pathologic changes occurring in animals fed selenium compounds are identical with those occurring in "alkalied" animals on the range and animals fed the toxic vegetation experimentally. In dogs, cats and rabbits treated with selenium compounds Czapek and Weil observed punctate hemorrhages on the pleura and epicardium and hyperemia and edema of the mucosa of the gastro-intestinal tract. Sodium selenite injected into rabbits, according to Jones, causes the liver to become soft and friable and produces distention of the right side of the heart, dilatation of the splanchnic vessels and a golden-brown deposit between the cells and commonly inside the cells of the viscera.

In experiments in which he fed rats toxic grains obtained from the selenized areas Franke found similar changes: Jaundice and emaciation, engorgement of the right auricle, congestion of the lungs and degeneration of the thymus, testes and ovaries are present. The mucosa, submucosa and muscularis of the stomach show hemorrhages with some small round cell infiltration, and the intestinal mucosa congestion. Early the liver is congested and later atrophied and hemorrhagic with extensive necrosis of the hepatic cells. In the regenerating stage hypertrophy is observed in the right lateral and caudate lobes and atrophy in the left lateral and central lobes of the liver; ascites and pleural effusion are commonly observed. The kidneys display hyperemia, degeneration and necrosis of the parenchyma with round cell infiltration. The convoluted tubules are particularly affected.

Selenium is concentrated in the liver, spleen and kidney in animals that have eaten seleniferous foods or that have been given selenium compounds experimentally (Dudley; Dudley and Byers; Jones; Duhamel and Juillard; Hofmeister and others). The heart, lung, brain and muscles contain small amounts. Dudley reported the element in the bile and hoofs of "alkalied" cattle.

RELATION OF OTHER ELEMENTS TO "ALKALI DISEASE"

Though the great bulk of evidence points to selenium as the cause of "alkali disease," the rôle of other elements, such as molybdenum, tellurium, arsenic, nickel, cobalt, tin, manganese, copper, vanadium and chromium, has been considered. Beath, Eppson and Gilbert found molybdenum in certain plants in appreciable quantities and with it produced symptoms resembling selenium poisoning in live stock. However,

Franke observed in selenium poisoning pathologic and symptomatic features that differ sharply from those produced by molybdenum, tellurium, arsenic and vanadium. At any rate, the significance of these elements, traces of which occur in arable soils, is deserving of further inquiry.

SUMMARY

Selenium poisoning, formerly called "alkali disease," has only recently come to the attention of students of animal diseases. The evidence indicates that "alkali disease" of live stock may be due to traces of selenium in indigenous grains and forage. Selenium is a relatively rare allotropic element having many of the physical and chemical properties of arsenic, sulfur and tellurium. It is widespread in the earth's crust, occurring in small quantities in the combined and free state, mostly associated with native sulfur, sulfate or metallic iron.

The disease is prevalent in the North Central Great Plains of the United States, effecting losses among cattle, horses, pigs, sheep and chickens which ingest selenium-bearing vegetation growing on arid or semiarid grazing lands and farms. The cereal-producing areas are involved, for the most part certain regions in South Dakota and Wyoming, and it is thought that North Dakota, Kansas, Montana, Nebraska, Colorado and New Mexico may be affected.

The cretaceous and eocene geologic formations are the selenium-bearing strata in this section of the country, particularly the Pierre formation, where from traces to 100 parts of selenium per million have been found. Soils derived from such strata and vegetation growing on such soils contain selenium in varying quantities. The total area of soils concerned is enormous, but not all the vegetation growing in these soils is toxic. Byers believes that seleniferous areas exist in at least four continents. All soils derived from pyritiferous parent materials bear selenium unless leeching has occurred. However, water in these toxic areas contains this element only in traces. Investigations have revealed the presence of selenium in many foods in the "alkalied" areas.

The toxicity of selenium compounds toward bacterial, plant and animal life varies with the ionic state. Selenite is more toxic than selenate, and reduced selenium is almost nontoxic. Selenium is readily absorbed but is rapidly reduced by all life. The toxicity of vegetation depends on its selenium content, and the absorptive power of plant life for selenium depends on the degree of rainfall, leeching, weathering, composition of the soil, the geologic outcrop on which the plant material grows, the species of plant and the amount of available sulfur or sulfate in the soil. Any soil containing more than 0.5 parts of selenium per million and any vegetation containing 5 parts per million is potentially dangerous (Byers). In the plant the selenium toxicant exists in

the protein fraction in an unknown organic combination and may be removed by mercuric chloride precipitation.

Whether animals are fed with inorganic selenium compounds or with the natural plant toxicant, the lesions and symptoms resemble closely those encountered among animals in the "alkalied" areas. Necrosis and regeneration of the hepatic parenchyma with extensive periportal fibrosis and cirrhosis combined with hemorrhages and secondary inflammatory reaction in the liver and kidneys are the outstanding lesions. Reduced selenium is found in the liver and spleen, and compounds of selenium are found in all tissues, secretions and excretions. Cattle, horses, sheep and pigs show erosions of the ends of the weight-bearing bones and abnormalities of the hoofs that considerably impair locomotion. Malaise, anorexia, stunting of growth, loss of body hair, loss of weight, emaciation, impairment of reproductive power and severe anemia develop, to be followed by exhaustion, toxemia and death. The poisoning may be acute and then is called "blind staggers." Chickens show a decided impairment of fertility; if the eggs hatch the young do not live long, and monstrosities occur among them frequently. Franke and his associates produced similar monstrosities in chicks by the injection of selenium salts into fertile eggs. The symptoms of experimental selenium poisoning in the guinea-pig and the white rat do not essentially differ from those in other animals.

Even if removed from the toxic range and fed wholesome forage, "alkalized" cattle do not usually recover their normal health.

The disease is essentially an agricultural and veterinary problem. The danger to human health seems to be relatively slight owing to the low content of selenium in the great portion of commercial crops and the high dilution of toxic grains in the general market. So far human selenium poisoning has not been reported in the toxic areas, but because of the occurrence of this malady in many varieties of animals there is no reason to believe that man is immune.

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Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Experimental Gastrectomy. H. B. Shumacker Jr. and M. M. Wintrobe, Bull. Johns Hopkins Hosp. **57**:384, 1935.

Totally gastrectomized dogs are extremely sensitive to carbon tetrachloride administered orally. This is believed to be due at least in part to the rapid absorption of the drug from the duodenum. In two totally gastrectomized dogs treated with small doses of carbon tetrachloride severe anemia developed, which was macrocytic in its later stages. Extensive damage of the liver was found at autopsy.

From the Authors' Summary.

Amniotic Fluid Concentrate as an Activator of Peritoneal Immunity. Herbert L. Johnson and others, Surg., Gynec. & Obst. **62**:171, 1936.

Guided by previous teachings and their own work, the authors have tried to reproduce aseptically the classic peritoneal reaction to infection. A variety of substances were employed as activators of peritoneal immunity, including amniotic fluid concentrate, physiologic solution of sodium chloride, papain and sodium ricinoleate. Of these, the first mentioned proved to be the most effective and innocuous. The experiments show that in dogs the character of the exudate depends on the substance introduced. It was found that agents relying on digestive ferments or excessive irritation interfere with the normal physiology of defense and repair. The brief interval of immunization required in the use of amniotic fluid concentrate makes it adaptable for operative as well as preoperative introduction.

Warren C. Hunter.

Carbon Tetrachloride Cirrhosis in Relation to Liver Regeneration. G. R. Cameron and W. A. E. Karunaratne, J. Path. & Bact. **42**:1, 1936.

Carbon tetrachloride in small amounts produces in the rat marked lobular degeneration and necrosis of the liver, followed at once by proliferation of the unaffected cells of the liver and complete repair in from one to two weeks. The smallest amount necessary to produce histologic evidence of damage in the rat's liver is about 0.025 cc. per kilogram of body weight, a dose within the limits of the therapeutic dose for man. Multiple exposures call forth in the rat's liver changes the nature of which is determined by the size of the dose and the interval between doses. A certain minimal amount, greater than the minimal toxic dose for the liver, must be given at short intervals to induce permanent effects. If there is time for recovery after each dose, carbon tetrachloride can be administered indefinitely without producing any permanent alteration. If, on the other hand, the time spacing does not allow complete recovery after each dose, cirrhosis of the liver results. There are two stages in the development of carbon tetrachloride cirrhosis: (1) a precirrhotic reversible stage with histologic features indistinguishable from actual cirrhosis and (2) a cirrhotic stage with a finely or coarsely granular liver. In the former, the liver reverts to normal on withdrawal of the drug. The restoration is due mainly to proliferation of liver cells, although it seems that fibrous tissue bands may actively disappear. The cirrhotic stage is characterized by the permanent nature of the perilobular fibrosis, which persists for months after the carbon tetrachloride has been withdrawn. There are also diminished capacity or absence of growth in the hepatic cells in autoplastic liver

grafts and impairment of restoration in the liver as a whole after partial hepatectomy. There is evidence also that in at least two other types of experimental cirrhosis (tar and sudan III) similar stages in the progress of the condition may be recognized.

FROM THE AUTHORS' SUMMARY.

EFFECT OF INJECTION OF PORPHYRIN IN EXPERIMENTAL RICKETS. E. EMMINGER and B. BÜCHELE, Virchows Arch. f. path. Anat. **295**:46, 1935.

The fact that porphyrin is deposited in the bones in congenital porphyria suggests a possible relation of this substance to calcium metabolism. Rats were rendered rachitic by a vitamin-free diet. The presence of rickets was determined by roentgenography. The animals then received daily subcutaneous injections of iso-uroporphyrin in doses of 0.5 mg. for varying periods up to twenty-two days. The animals were kept in the dark. Porphyrin was present in the original bone and in new-formed bone but not in the osteoid tissue or cartilage. There was some evidence of healing of the rickets. The healing is held to have been spontaneous and not due to the porphyrin.

O. T. SCHULTZ.

Pathologic Anatomy

LESIONS IN THE ROOTS OF THE PULMONARY ARTERY AND AORTA IN RHEUMATIC FEVER. L. GROSS, Am. J. Path. **11**:631, 1935.

A variety of lesions found in the roots of the pulmonary artery and aorta together with their pericardial mantles in rheumatic fever have been described and considered statistically. The observations were made on sixty-six actively rheumatic hearts, thirty-four inactively rheumatic hearts and fifty nonrheumatic control hearts. It is shown that in both active and inactive rheumatic fever the aortic and pulmonic roots display a strikingly high incidence of destructive and inflammatory lesions such as scarring, disruption of the elastica and vascularization. It is also shown that the pericardial mantles surrounding the roots of these great vessels display a high incidence of various vascular lesions. Some of these are similar to those occurring in nonrheumatic controls, due to the changes related to age. Others, however, are similar to the vascular lesions due to rheumatic fever found in the myocardial coronary ramifications. A discussion is given of the significance of these observations.

FROM THE AUTHOR'S SUMMARY.

CHRONIC PULMONARY ARTERITIS IN SCHISTOSOMIASIS MANSONI. E. CLARK and I. GRAEF, Am. J. Path. **11**:693, 1935.

A case of schistosomiasis Mansoni in a young Puerto Rican dying of congestive heart failure is recorded. Hypertrophy of the right ventricle, dilatation of the pulmonary artery and lesions of the small pulmonary arteries are described, and it is demonstrated that the latter represent a form of specific arterial disease caused by the presence of ova of Schistosomum Mansoni.

FROM THE AUTHORS' SUMMARY.

PULMONARY EMBOLISM FOLLOWING TRAUMA. J. S. MCCARTNEY, Surg., Gynec. & Obst. **61**:369, 1935.

Only a few extensive reviews of posttraumatic pulmonary embolism are found in the literature, and none have appeared since 1913. The present analysis is based on 1,499 instances of death from mechanical injury. In this group the incidence of pulmonary embolism was 4 per cent. Although males were injured more than three times as frequently as females, the incidence of embolism was 8.6 per cent in the females and only 2.8 per cent in the males. In about 80 per cent of the cases of posttraumatic pulmonary embolisms there was injury of a lower extremity, usually a simple fracture. Embolism was rare after fractures of bones other than those of the lower extremity. The difference in incidence in the sexes could not be

explained on the basis of age, cardiac disease or the time of death after injury. McCartney suggests that the higher incidence of varicose veins in women may be the key to the situation.

WARREN C. HUNTER.

CHRONIC ATROPHIC DERMATITIS OF THE VULVA. F. L. ADAIR and M. E. DAVIS, Surg., Gynec. & Obst. **61**:433, 1935.

The descriptive term employed here is preferable to any of the many names hitherto proposed because it indicates the nature of the entire process rather than emphasizes only certain phases of the condition. Among gynecological patients seen by the writers the incidence of the lesion at one stage or another was 0.24 per cent. It is a progressive disease, and unless vulvectomy is performed, leads to carcinoma in more than half of the cases. The three stages of the lesion are excellently depicted in colored plates.

WARREN C. HUNTER.

THE CARBONIC ANHYDRASE CONTENT OF BLOOD IN PATHOLOGICAL STATES IN MAN. T. H. HODGSON, Brit. J. Exper. Path. **17**:75, 1936.

The absolute amount of carbonic anhydrase in the blood is decreased in anemia and increased in cyanosis. There is usually a rough correspondence between the amount of carbonic anhydrase and the percentage of corpuscles as given by the hematocrit. Exceptions to this relation were observed notably in cases of jaundice.

FROM THE AUTHOR'S SUMMARY.

THE NATURE OF LOCALIZED FIBROUS OSTEITIS. S. MUSTAKALLIO, Arb. a. d. path. Inst. d. Univ. Helsingfors **8**:38, 1935.

Localized fibrous osteitis can be classified into five groups: bone cyst, benign giant cell tumor in the bone, central bone fibroma, osteofibroma and transitional forms. The groups are similar in that the growth begins in the bone, which is destroyed progressively toward the periphery while the periosteum simultaneously builds new bone on the surface. The periosteum always remains in direct connection with the new tissue. In all cases one deals with a mesenchymal structure in which regions of collagenous connective tissue predominate. In most cases there are regions in which the cells show a comparatively low grade of differentiation. In these cells there are relatively many mitoses. These are obviously centers of growth. In the bone cyst these centers line the wall of the cyst or of its projections. In the benign giant cell tumor the centers of growth are more widely distributed in the middle part of the tumor. In the central bone fibroma, the growth takes place in the wall of the cyst when cysts are present. In the solid form the areas of growth are diffuse but most marked in the central portions of the tumor. This is true of the last two groups also. The number of mitoses decreases from the center of growth toward the periphery, and the cells gradually become differentiated into fibroblasts and cells resembling fibrocytes. Giant cells are most numerous in the second group and rather sparse in the first. In the third group they are rare or absent. In the fourth group they are absent. Giant cells arise from fusion of cells. It is possible that except in trinucleate cells the increase in nuclei is accomplished through mitotic or amitotic division. Around the edges the osteoclasts arise from the same elements as the osteoblasts. The division of giant cells into cells of a single nucleus cannot be established, and there is no evidence to show that giant cells are formed from endothelial cells. Osteoblasts and bone cells are not specific for any group. If one has a "juxtanuclear vacuole" it is certain that the cell is forming bone. Independent bone formation of two types may proceed from the periosteum. In the first, homogeneous masses are laid down between the fibers in any part of the collagenous connective tissue, and immediately thereafter, on the surfaces of this mass, cells appear which resemble osteoblasts without "juxtanuclear vacuoles." These vacuoles can be found later if bone formation proceeds simultaneously. In the second type of bone formation, amorphous bodies

the size of cells appear between the bands of connective tissue and are arranged in adjacent groups. The osteoblasts then develop on the surface of these groups, apparently from fibroblasts, for the transitional forms of all stages are found. The nuclei of the cells in the center of growth are larger, in general, than the nuclei of the cells of the granulation tissue. Often amorphous or hyaline masses appear as intercellular substance. Principally on the basis of these observations and of the clinical picture, the conclusion is reached that these formations are to be considered, at most, as benign neoplasms. They form a continuous sequence of tumors from fibroma of a high grade of differentiation to fibrosarcoma.

From the Author's Summary. (Elizabeth McBroom.)

Microbiology and Parasitology

Cutaneous Torulosis. Udo J. Wile, Arch. Dermat. & Syph. **31**:58, 1935.

A fatal case of disseminated torulosis in a boy aged 17 is presented. Swelling of the neck had been noticed for four and a half years. When it first appeared a cervical gland was removed and a diagnosis of Hodgkin's disease was made. On the thighs and behind the knees were irregular patches of boardlike hardness, appearing like ecchymoses or bruises. Biopsy showed the deeper layers of the dermis and subdermis to be infiltrated with innumerable giant cells. These were in various degrees of degeneration and contained enormous numbers of refractive organisms having a double contour. Many lymphocytes and fibroblasts were also present. There was no suppuration. Reexamination of microscopic sections of the gland that had been removed showed, in addition to changes resembling Hodgkin's disease or lymphosarcoma, scattered double-contoured yeastlike bodies of the same type as were found in all the organs at autopsy. It was considered, therefore, that the torula had produced the original swelling.

S. W. Becker.

Bacterium Necrophorum in Chronic Ulcerative Colitis. G. M. Dack, L. R. Dragstedt and T. E. Heinz, J. A. M. A. **106**:7, 1936.

Bacterium necrophorum was found to be the predominant type of organism in the isolated colons of three patients with severe nonspecific ulcerative colitis. It persisted as the predominant type as long as the colons remained severely diseased and became less prominent during periods of remission. It was isolated by appropriate methods from the nonisolated colons of seven of twelve additional patients with nonspecific chronic ulcerative colitis and two patients with specific ulcerative colitis. Complement-fixing antibodies for Bact. necrophorum were found in the serum of fourteen of sixteen patients with typical chronic ulcerative colitis and in that of only three of sixteen control patients. These facts together with the abundant evidence in the literature supporting the claims that this organism is pathogenic for lower animals and man have led the authors to consider it as of etiologic significance in chronic ulcerative colitis. From the Authors' Summary.

Pathology of Pneumococcus Infection in Mice Following Intranasal Instillation. G. Rake, J. Exper. Med. **63**:17, 1936.

Pneumonia can be produced in mice which have not been previously prepared, by intranasal inoculation of broth cultures of certain strains of pneumococci. Inoculation of pneumococci of the same type in different breeds of mice produces lesions which are quantitatively different. Inoculation of pneumococci of different types into one breed of mice results in lesions which are qualitatively different. In general, the qualitatively different lesions were noted as follows: diffuse pneumonia and acute glomerular nephritis in unselected mice receiving type I strains; confluent pneumonia and tubular nephritis in those receiving type II strains, and interstitial pneumonia with extensive gelatinous pleurisy together with necrosis and abscess formation in the spleen and cervical lymph nodes in those receiving type

III strains. Resistant strains of mice inoculated with type III pneumococci show slight changes in the lungs but marked lesions in the spleen and cervical nodes, while susceptible mice inoculated with pneumococci of the same type show marked changes in the lungs and moderate lesions in the spleen and cervical nodes. The development of type III pneumonia, studied by means of serial sections of nasally infected mice, appears to proceed in the stages of vascular engorgement, interalveolar interstitial exudation, exudation of albuminous fluid into the alveoli and the perivascular lymphatics draining the affected site and finally frank pneumonia with an exudation of cells, but without much fibrin, into the alveoli.

FROM THE AUTHOR'S SUMMARY.

THE AUSTRALIAN EPIDEMIC OF ENCEPHALOMYELITIS. J. R. PERDRAU, J. Path. & Bact. **42**:59, 1936.

The Australian X disease studied by Cleland and Campbell was caused by a virus with characteristics so closely resembling those of the virus of louping ill that differentiation between them on the evidence now available is well nigh impossible, but no claim is made that they are one and the same virus.

FROM THE AUTHOR'S CONCLUSION.

TUBERCULOUS MENINGITIS. A. BOUQUET and R. BROCA, Ann. Inst. Pasteur **55**: 8, 1935.

Suboccipital injection of bovine tubercle bacilli into rabbits and guinea-pigs produced fatal meningitis. Tubercle bacilli of the human type were less virulent, but those of the avian type approached in virulence the bovine variety. The latter induced minor macroscopic lesions of an inflammatory order. No matter what the type of the bacilli, the meningitis paved the way to encephalitis or encephalomyelitis. In large doses dead organisms also produced fatal meningitis. The possibility of superinfection suggested that the brain did not react in the same way as other tissues. The authors were not successful in producing tuberculous meningitis by intravenous injection, even when an aseptic meningeal reaction was artificially produced.

THE ETIOLOGY OF INFLUENZA. M. ISABOLINSKI and N. STRATANOWITSCH, Zentralbl. f. Bakt. (Abt. 1) **133**:175, 1935.

Cultures obtained from the nasal passages of patients in the first days of influenza yielded both diplococci and Pfeiffer's bacilli. The latter were quite pleomorphic, ranging from fine long rods to coccobacilli. These organisms when instilled into the nostrils of rabbits induced acute inflammation, and pure cultures were recovered from the inflamed tissues. The serum of the infected rabbits contained agglutinins and complement-fixation antibodies for Pfeiffer's bacilli. The organisms when injected intraperitoneally into young guinea-pigs caused death, and a pure culture of the bacilli could be obtained from the blood. The authors suggest that the diplococci so frequently recovered from the nasal passages of patients with influenza may be variants of Pfeiffer's bacilli.

PAUL R. CANNON.

CARRIERS OF BACTERIOPHAGE AMONG BACTERIA. F. HODER and R. AKANO, Ztschr. f. Immunitätsforsch. u. exper. Therap. **85**:423, 1935.

Hoder continued his studies on the ability of some bacterial strains to carry bacteriophage without themselves presenting any effects of that association. Five such carriers were found among twenty-five strains of E-dysentery bacilli (Kruse-Sonne). These bacteriophages were strictly specific. Five carriers were found among forty strains of Staphylococcus aureus from human sources. These five bacteriophages were less specific; they lysed also certain saprophytic cocci from

the air and soil. Two of forty cocci isolated from the soil were carriers of phages that lysed only Staph. aureus from human sources. No carriers were found among ninety-five strains of bacteria that were isolated from the air.

I. DAVIDSOHN.

Immunology

PURIFICATION OF THE ANTIBODIES IN TYPE I ANTIPNEUMOCOCCUS SERUM AND THE CHEMICAL NATURE OF THE TYPE-SPECIFIC PRECIPITIN REACTION. B. F. CHOW and W. F. GOEBEL, J. Exper. Med. **62**:179, 1935.

It is believed that the general mechanism underlying the union of antibody and carbohydrate involves the interaction of polar groups of opposite charges. Similar views have indeed been expressed by Heidelberger and Kendall, by Marrack and by Breinl and Haurowitz. In the case of the carbohydrate of Pneumococcus type I it is believed, on the basis of the evidence presented, that the carboxyl groups of the polysaccharide are the dominant groups which interact to form the immune precipitate. Whether the carboxyl groups actually combine with the amino groups of the antibody protein and whether the formation of an insoluble precipitate involves further chemical change in the protein molecule, such as a specific denaturization, cannot be answered with certainty at the present time. It is believed, however, that the specificity of this reaction is determined by the stereochemical relationship of the dominant polar groups in the reacting molecules, whether they are antigen or antibody. If the spatial pattern of the polar groups of both antigen and antibody is of exactly the correct order, union occurs. If, however, this relationship is disturbed by artificial means, as has been experimentally demonstrated by covering the dominant polar group of either polysaccharide or antibody with a chemical radical, the pattern is destroyed, and union between them is either greatly modified or fails to take place. When the original constitution of the reacting substances is restored, however, serologic specificity is regained.

THE RELATIVE IMPORTANCE OF RETICULO-ENDOTHELIAL TISSUES AND CIRCULATING ANTIBODY IN IMMUNITY. F. H. TEALE, J. Immunol. **28**:133 and 161, 1935.

Virulent and nonvirulent bacteria (Bacillus dysenteriae Shiga, Pasteurella of chicken cholera, Bacillus anthracis, Streptococcus, Pasteurella canis, Staphylococcus) when injected intravenously into rabbits were readily cleared from their circulation, although the blood of the rabbits lacked agglutinating and germicidal ability as well as the property to protect animals by passive immunization. The removal of intravenously injected anthrax bacilli which were obtained from the peritoneal exudate of animals dying as a result of a fatal injection was slow as compared with the disposal of bacteria of the same type grown on culture mediums The bacteria that were removed from the peripheral circulation were found enmeshed in fibrin and in blood platelets within the capillaries of the large central organs. The rôle of physical factors is evidenced by the fact that injection of heparin together with the bacteria slowed up considerably the disappearance of the bacteria from the peripheral circulation. Virulent pneumococci were the only bacteria that were not cleared from the peripheral circulation by nonimmune rabbits, but properly immunized animals cleared them readily, although their blood had no agglutinating, germicidal or protective antibodies. The rabbits retained the clearing property with regard to the pneumococci for at least twelve months after immunization. Rabbits immunized with Friedlander's bacillus behaved like those treated with pneumococci. A definite amount of antibody must be present in a serum in order to produce passive hypersensitiveness. In the case of lytic antibodies, there is a direct relation between their titer and their ability to sensitize; the number of lytic units required for sensitization varies with the different antigens. No direct relation was noted between the precipitins and the sensitizing power of immune serums. It does not matter whether the sensitizing

serum comes from an animal of the same species or not. The hemolytic serums that are difficult to produce have a greater sensitizing power than those which are easily produced (for instance, antihuman lysins as compared with antisheep lysins). There is no special sensitizing antibody; the removal of the lytic antibody destroys the sensitizing property. The injection into a hypersensitive animal of the homologous antigen that has become thoroughly saturated with the specific antibody does not prevent shock. Teale concludes from his experiments that circulating antibodies play a relatively insignificant rôle in immunity. The tissues are the main factors.

I. DAVIDSOHN.

SIMULTANEOUS IMMUNIZATION WITH A MIXTURE OF TEN KINDS OF LAKED BLOOD. E. DELVES, J. Infect. Dis. **57**:61, 1935.

Precipitins for many blood proteins and for agglutinins and lysins for different species of blood corpuscles were developed simultaneously in the blood of animals immunized with a mixture of ten kinds of laked blood. Absorption experiments showed that the precipitins for hemoglobin and the agglutinins and hemolysins for red blood cells must be produced by two separate antigens since they can be absorbed specifically.

FROM THE AUTHOR'S SUMMARY.

ANTIGENIC PROPERTIES OF GLOBINS. C. A. JOHNSON and W. B. BRADLEY, J. Infect. Dis. **57**:70, 1935.

Globin prepared in the manner described is a strong antigen which exhibits the same species specificity as hemoglobin. Globin is absorbed on aluminum hydroxide and in this respect differs from hemoglobin. When globin is used as an antigen, it evokes precipitins in the antiserum which appear to be identical with those produced when hemoglobin of the same species is injected. Hence, it appears that globin is responsible for the antigenic properties of hemoglobin. Species specificity is apparently due to inherent differences in the structure of the various globins.

FROM THE AUTHORS' SUMMARY.

THE TOXICITY OF FILTRATES OF BROTH CULTURES OF MENINGOCOCCI. B. G. MAEGRAITH, Brit. J. Exper. Path. **16**:109, 1935.

Filtrates of three to six day cultures of meningococci of both groups I-III and II contain substances toxic to guinea-pigs, mice and rabbits. These substances are best demonstrated by subdural introduction of the filtrate through the occipital bone of the animal into the subarachnoid space. The action of the filtrates on animals is very similar to that of various so-called endotoxins produced directly from meningococcic cells. There are, however, histologic differences which suggest that the toxic filtrates contain substances not present in the cell extracts.

FROM THE AUTHOR'S SUMMARY.

THE ADSORPTION OF ANTIBODIES FROM SYPHILITIC AND TUBERCULOUS SERUM. G. D'ALESSANDRO and F. SOFIA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**:237, 1935.

When kaolin or charcoal was added to an alcoholic extract of beef heart as it is used in the Wassermann test and the mixture centrifugated, and then the serum of a syphilitic patient was treated with either of the two preparations, the corresponding antibodies were removed and the positive Wassermann test turned negative. The same observations were made when the Wassermann antigen and the adsorbents were added separately to the syphilitic serum. The precipitating antibodies were adsorbed together with the complement-fixing properties. Similar treatment of a tuberculous serum with the homologous antigen prepared according to the method of Witebsky brought about a complete removal of the specific complement-fixing antibody. The specificity of the reaction was demonstrated by separate removal of the corresponding antibodies from the serum of a patient who

was afflicted with syphilis and tuberculosis simultaneously. Addition of kaolin or of charcoal alone did not influence the titer of the antibodies, but each was able to remove a considerable quantity of the antibodies if alcohol was added to the serum. That the presence of the alcohol was not a deciding factor in the described adsorptions with the extracts of beef heart is evidenced by the fact that when the alcoholic extract was evaporated and the sediment resuspended in physiologic solution of sodium chloride the removal of the antibodies was equally complete.

I. DAVIDSOHN.

THE DURATION OF PASSIVE IMMUNITY. A. STRØM, Acta path. et microbiol. Scandinav. **12**:275, 1935.

The experiments reported have confirmed the findings of Glenny and Hopkins that in rabbits following an injection of 2,000 units of diphtheria antitoxin the antitoxin disappears from the blood in from eight to ten days. Adding aluminum hydroxide or fractionating the dose does not prolong the period in which the antitoxin can be detected in the blood. For the rapid disappearance of the antitoxin, precipitins presumably are of great importance. The finding of a short interval between the beginning of the most rapid loss of antitoxin and the appearance of precipitins is in accordance with the results of Madsen.

Tumors

THE ORIGIN OF MIXED TUMORS OF THE SALIVARY GLANDS. P. L. LI and C-S. YANG, Am. J. Cancer **25**:259, 1935.

Mixed tumors are probably embryonic tumors of local origin. From histologic, regional and histogenetic points of view, they seem naturally to fall into two groups: intra-oral and extra-oral. To the first group belong tumors arising from oral ectoderm which lie within the oral cavity, including tumors of the palate, gum, tongue, salivary glands, etc. The embryonic rests from which these are derived are formed along with the salivary and oral glands from invagination of the oral ectoderm as assumed by Wilms. To the second group belong tumors of extra-oral origin: (*a*) tumors resulting from ectodermal inclusions in the course of the fusion of branchial clefts and various fissures of the head and neck; (*b*) tumors of the nasal cavity and its accessory sinuses arising from rests formed along with the normal mucous glands of these regions; (*c*) tumors of the orbit derived from rests formed from the conjunctiva together with the anlagen of the lacrimal glands; (*d*) various other mixed tumors of the lip, face, eyebrow, scalp, etc., assumed to have arisen from rests derived from the integument along with the anlagen of the hair follicles. The histologic similarities and differences among the mixed tumors and tumors closely allied to them are explained by differences in the origin and time of the formation of the rests from which they are derived. The location and the frequency of occurrence are explained on the same ground. The influence of the time factor in the formation of embryonic rests on their expected potentiality is especially emphasized.

FROM THE AUTHORS' SUMMARY.

DYSONTOGENETIC ORIGIN OF BASAL CELL CARCINOMA. J. MCFARLAND, E. F. CICCONE and J. GELEHRTER, Am. J. Cancer **25**:273, 1935.

A substantial number of carefully selected and histologically confirmed basal cell carcinomas from two different hospitals have been plotted on two diagrams of the human face in order that their distribution might be studied with reference to their possible dysontogenetic origin through defective concrescence of the embryonal facial fissures as suggested by Glasunow. The results have been compared with the plottings of other supposed dysontogenetic lesions, sequestration dermoids and mixed tumors, with the result that all three types of lesion are found to conform to about the same anatomic distribution, which is entirely different from that of

the more common squamous cell or prickle cell carcinomas. It is not believed that the observations presented settled the question, but it is believed that they support the theory of Glasunow that the basal cell carcinomas are dysontogenetic tumors which originate in imperfections in the closure of the embryonal facial fissures.

FROM THE AUTHORS' CONCLUSIONS.

DEVELOPMENT OF THE MAMMARY GLANDS OF VIRGIN FEMALE MICE OF TEN STRAINS VARYING IN SUSCEPTIBILITY TO SPONTANEOUS NEOPLASMS. W. U. GARDNER and L. C. STRONG, Am. J. Cancer **25**:282, 1935.

This study indicates that no structural factor in the development of the mammary glands in virgin female mice was associated with the intrinsic hereditary predisposition to mammary carcinoma in mice.

FROM THE AUTHORS' CONCLUSIONS.

ESTROUS CYCLES OF MICE WITH SPONTANEOUS MAMMARY TUMORS. E. ALLEN and OTHERS, Am. J. Cancer **25**:291, 1935.

Estrous cycles were studied in fifty-nine mice of four different strains inbred with regard to early appearance of spontaneous mammary tumors. The types of tumor involved were mammary carcinoma and adenoma. With advanced growth of the tumors estrous cycles became infrequent and long continued periods of diestrum occurred. Sections of the genital organs showed them to be in an atropic condition approaching that of the same organs in ovariectomized animals. After long periods of diestrum the accessory genital organs were stimulated repeatedly to normal estrous activity by injections of theelin and by implants of two or three pituitaries from gonadectomized mice. Several successive treatments with either theelin or anterior pituitary or combinations of both were tried. There was found a time in the degenerative phase of the ovary when pituitary treatment was no longer effective. Minimal doses of theelin produced positive vaginal reactions in such animals. These results indicate that the acyclic condition in mice with mammary tumors probably involves primarily the gonadotropic function of the pituitary.

FROM THE AUTHORS' SUMMARY.

LEUKOCYTIC INVASION AS AN ACCOMPANIMENT OF EPITHELIAL METAPLASIA. H. BURROWS, J. Path. & Bact. **41**:43, 1935.

When an epithelium which does not normally form keratin is converted into a keratinizing one, an invasion by polymorphonuclear leukocytes is apt to occur. Leukocytic invasion may be observed in the vagina and uterus of the female mouse in the course of the natural estrous cycle, in the genital organs of the male mouse under continued treatment with estrogenic substance and in various structures in rats which have been maintained on a diet deficient in vitamin A. Reasons are given for supposing that the leukocytic invasion in these conditions is a physiologic process and directly attributable neither to the presence of desquamated keratinous material nor to bacterial infection.

FROM THE AUTHOR'S SUMMARY.

THE RATE OF TUMOR INDUCTION BY TAR IN THE MOUSE AT DIFFERENT SITES IN THE SKIN. J. W. ORR, J. Path. & Bact. **41**:51, 1935.

When mice are tarred in the scapular and sacral regions of the skin of the back, the tumors which occur at each site may appear at any time during the period of tumor incidence for that site, irrespective of the changes occurring at the other site. Early or late scapular tumors do not predicate early or late sacral tumors, and vice versa. In comparing the relative carcinogenic potency of different procedures, the study of one area of scapular skin in groups of sufficient magnitude is a more sensitive index than the comparison of the scapular and sacral areas in the same animal.

FROM THE AUTHOR'S SUMMARY.

PAPILLARY CYSTO-ADENOLYMPHOMA. J. F. TELLO and J. M. HERRERA, Trav. du lab. de recherches biol. de l'Univ. de Madrid **29**:157, 1934.

An unusual tumor removed from the carotid region of a 45 year old man is described. It was a fibrous encapsulated growth with multiple cystic cavities, some of which contained a mucus-like fluid, others a hemorrhagic accumulation. The tumor was composed of papillomatous excrescences in cystic cavities, covered by columnar epithelium. The stroma was composed of typical lymphoid tissue. It was impossible to determine the origin of the tumor. The literature contains reports of five similar cases.

CYRIL B. COURVILLE.

EXTRAGENITAL CHORIONEPITHELIOMA IN A MAN WITH A POSITIVE ASCHHEIM-ZONDEK TEST. E. FENSTER, Frankfurt. Ztschr. f. Path. **46**:403, 1934.

The autopsy on a 28 year old man revealed in the retroperitoneal region a large tumor attached to the spinal column, liver, pancreas, left adrenal, left kidney, stomach and proximal portion of the duodenum. The inferior vena cava and the abdominal aorta were compressed. Metastases were seen in the lungs, liver and peritracheal lymph nodes. Histologically the tumor consisted of much necrosis and many large polygonal cells with nuclei that were rich in chromatin and varied greatly in size. Many mitotic figures were seen. In many fields the tumor cells were arranged in glandular fashion. Syncytial cells were commonly encountered containing from 3 to 15 nuclei and were similar to those seen in the typical chorionepithelioma of the uterus. Serial sections through both testes did not reveal a tumor. The Aschheim-Zondek test was positive with material taken from the primary tumor and from the metastases in the lung and liver. Fenster stresses that gynecomastia was not present and that the localization of the metastases of the extragenital and the testicular chorionepithelioma is the same.

OTTO SAPHIR.

Medicolegal Pathology

CHANGES IN THE EPENDYMAL AND SUBEPENDYMAL ZONES OF THE BRAIN CAUSED BY RECENT HEAD INJURIES. J. GIERLICH, Deutsche Ztschr. f. d. ges. gerichtl. Med. **26**:100, 1936.

Changes in the walls of the cerebral ventricles and in their vicinity were found in a large percentage of cases of cranial injury by blunt force. The changes were in the form of ependymal defects, hemorrhages about the defective and intact ependyma, either along the flat wall or the poles of the ventricles, and warty bulgings of the subependymal glia. The changes were ascribed to the alteration of hydrodynamic pressure within the ventricles occasioned by the trauma. In the presence of alcoholic intoxication or arteriosclerosis the ependymal injury was always extensive, even after minor trauma. The greatest part of the centrally located change was not visible to the naked eye and therefore had to be sought in the deeper zones removed from the direct or contrecoup lesions. Age seemed to play no factor in the condition, since the lesions were equally severe in children and adults. Fatty changes were frequently present in the ependymal cells, even in persons who died immediately after their injury.

GEORGE RUKSTINAT.

INTRACRANIAL HEMORRHAGE AFTER HEAD INJURY WITHOUT SKULL FRACTURE. G. JUNGMICHEL, Deutsche Ztschr. f. d. ges. gerichtl. Med. **26**:130, 1936.

Intracranial hemorrhage was encountered in 5.6 per cent of persons who had head injuries but no skull fractures. Jungmichel explains extradural hemorrhage without skull fracture in young persons on the basis of cranial elasticity—the bone being able to bend under a blow. The tight dura, however, is torn from its bony bed by the blow, and a tear in the vessels on its exterior surface results in hemorrhage. The bleeding is primarily from arteries, the accompanying veins contribut-

ing only slightly. Fissures of the skull must be ruled out by roentgen examination, postmortem inspection and, if necessary, maceration of the suspected bone.

Subdural hemorrhage occurs from arteries, large venous sinuses and torn meningeal veins. Only slight trauma is necessary to induce such bleeding, and there is no basis for the statement that such accidents occur typically only in boxers. Similar lesions may be found in the football player struck on the head with a rain-soaked ball.

The intracerebral lesions were various and at times seemed associated with the site of action of the direct force; at other times contrecoup lesions were apparent. Some of the injuries seemed dependent on the glancing character of a blow; others, on the transmission of the force wave through the fluid in the lateral ventricles.

GEORGE RUKSTINAT.

ABSORPTION OF ALCOHOL AFTER INGESTION OF FOOD. H. ELBEL and G. LIECK, Deutsche Ztschr. f. d. ges. gerichtl. Med. **26**:270, 1936.

If an alcoholic beverage is drunk after taking in food the concentration of alcohol in the blood is considerably lower than if the same amount of the beverage is taken on an empty stomach. This difference is due essentially to delay in absorption of the alcohol, which depends on the quantity and probably on the digestibility of the food.

FROM THE AUTHORS' SUMMARY.

CHANGES IN THE ALCOHOL CONTENT OF CADAVER BLOOD AND OF BLOOD SAMPLES PRESERVED WITHOUT STERILE PRECAUTIONS. K. WAGNER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **26**:276, 1936.

The amount of alcohol in the blood of the human cadaver diminishes during the first two days after death on an average of 5 to 6 per cent and during the first four days about 20 to 25 per cent. Samples of blood taken from cadavers later than four days after death are not usable, because the products of decomposition may give rise to an apparently high alcoholic content. The blood in different parts of the cadaver may contain the same quantity of alcohol, but as a rule the blood from the head and the heart gives the highest values. Blood preserved without special precautions may be used for the determination of its alcoholic content only up to the fourth day, for in the following days considerable diminution in the alcoholic content takes place. In blood free from alcohol and preserved under sterile precautions reducing substances which may lead to simulation of the presence of alcohol are not demonstrable during the first four days.

Society Transactions

PATHOLOGICAL SOCIETY OF EASTERN NEW YORK

ARTHUR W. WRIGHT, *Secretary*

Regular Meeting, Jan. 10, 1936

M. CRISCITIELLO, M.D., *Presiding*

ATROPHIC CIRRHOSIS OF THE LIVER WITH MYELOGENOUS LEUKEMIA. G. H. KLINCK JR.

A white man 64 years of age, employed as a signal man, was treated at home for "pneumonia." He was convalescing unusually slowly when pains in the upper part of the abdomen, drowsiness, occasional spells of vomiting and unilateral acute parotitis developed. Because of these conditions he was brought to the hospital, where it was discovered that he had uremia, with the nonprotein nitrogen of the blood 130 mg. and the creatinine 3.6 mg. per hundred cubic centimeters. The urine contained much albumin. The temperature was 99 F. on admission but rose to 106 within a few hours. The patient became weak and comatose and died thirty-two hours after admission.

Autopsy disclosed: diffuse bilateral bronchopneumonia with several bronchiectatic cavities in the right lung; a spleen which weighed 950 Gm.; an enlarged sclerosed liver; nephrolithiasis; leukemic infiltrations of the kidneys; generalized hypertrophy of the lymph nodes, and hyperplasia of the bone marrow.

Microscopically the splenic pulp, hepatic sinuses and renal cortices were infiltrated with large numbers of atypical myeloid cells and many megakaryocytes. The cells were definitely neoplastic. In the kidneys these cells were collected into nodular masses. The fatty marrow of the femur was completely replaced by neoplastic cells of the leukopoietic system. Myeloblasts and myelocytes predominated. The sinuses of the abdominal lymph nodes were infiltrated with cells of the same type. The liver showed early portal cirrhosis of the toxic type, and the kidneys were the seat of subacute and chronic pyelonephritis of rather long standing.

The final diagnosis was myelogenous leukemia with concomitant cirrhosis of the liver, chronic pyelonephritis and acute parotitis.

The patient was in the hospital for a little more than one day. No studies of the blood were made, and there was no clinical suggestion of leukemia. The presence of cirrhosis was not appreciated in the absence of signs of portal obstruction. The case emphasizes particularly the importance of early routine studies of the blood of any person admitted to the hospital.

ARGENTAFFIN TUMOR OF THE APPENDIX IN A PREGNANT WOMAN. J. SCHLEIFSTEIN.

The literature on carcinoid or argentaffin tumor of the appendix is extensive. Masson, Forbus, Raifford and Smith have clearly described various features of this type of neoplasm. The case presented differs from those previously reported in that the tumor was removed during pregnancy.

A woman aged 40 complained of backache, abdominal pain, menorrhagia and metrorrhagia of two months' duration. Physical examination showed an enlarged soft uterus. Appendectomy and hysterectomy were performed.

The appendix was firm, about the size of a pigeon's egg, and somewhat curled on itself. On section, solid yellowish tumor masses were seen invading and replacing the tissue.

On microscopic examination the characteristic structure of the organ was found to have entirely disappeared, and the lumen was completely obliterated. Compact masses of epithelial cells were grouped in nests and strands, which were particularly prominent in the center of the section and extended throughout all the coats of the appendix. A dense stroma, probably hypertrophied muscularis, separated these clusters. An occasional pseudo-alveolar arrangement was also observed. The lymphatics were extensively invaded. The tumor was composed of rounded cells with well stained nuclei and scanty cytoplasm. A few mitoses were noted. The neoplasm had spread to the serosa and probably farther. The observations correspond with the classic description of the argentaffin type of tumor of the appendix.

Sections of the uterus showed evidence of pregnancy. The cervix was chronically inflamed and cystic.

MUCOCELE OF THE APPENDIX. M. CRISCITIELLO.

A 20 year old woman complained of a sense of fulness in the right lower quadrant of the abdomen. Her appetite was poor. There was moderate spasm of the abdominal wall, and a mass the size of a lemon was felt on deep palpation. The temperature was 98.6 F. The leukocytes numbered 10,900, 81 per cent of which were polymorphonuclear leukocytes. A diagnosis of acute appendicitis with probable abscess was made.

Operation disclosed an enlarged appendix, half of which was invaginated into the cecum to form a tumor-like mass. Periappendical adhesions were present, and the enlarged organ had to be dissected out. During this procedure it ruptured, liberating much jelly-like mucoid material.

The patient recovered uneventfully.

The appendix was 11 cm. long. Its proximal third was cystic and ruptured. It had a thin membranous wall. The cyst still contained some mucoid material. A small focus of calcification was present in the wall near the point of excision. The distal portion was congested, thick-walled and not dilated. Much mucoid material was received along with the organ.

In a series of 31,200 cases in which appendectomy was done at the Mayo Clinic from 1917 to 1930 there were 76 in which mucocele was found, an incidence of 0.25 per cent. The condition apparently results from complete obliteration of the lumen at one point associated with active secretion on the part of the mucous membrane, which produces more mucus than the organ can absorb. There is also a change in cellular activity whereby pseudomucin rather than normal mucus is formed. The most serious complication of this condition is the production of pseudomyxoma peritonaei, which resembles that following the rupture of a pseudomucinous cyst of the ovary. The prognosis in a condition of the latter type, however, is considered to be poorer than that in pseudomyxoma peritonaei following mucocele of the appendix. The possibility of this complication occurring in the present patient cannot be ruled out.

LYMPHOSARCOMA OF THE TESTES. V. W. BERGSTROM.

An Italian 39 years of age first noticed a painful lump in the right side of the neck three weeks before he came to the hospital. Gradually more masses appeared as other lymph nodes were involved. The testes also became swollen. The patient had had syphilis in 1923 and had suffered from diabetes for several years.

At the time of admission there was glandular enlargement in both sides of the neck, and the testes were enlarged. Girdle pains were present at the level of the

twelfth dorsal vertebra, and numbness of the left arm was noted. There was a dry cough associated with slight dyspnea and pain in the chest. The patient had lost much weight.

The temperature was normal. The Wassermann reaction of the blood was positive. The nonprotein nitrogen of the blood was 117 mg., and the sugar was never lower than 147 mg. per hundred cubic centimeters. Roentgen examination of the thorax showed a shadow in the lower lobe of the left lung, enlargement of the heart and absence of skeletal lesions. Histologic examination of an excised lymph node revealed a tumor which was diagnosed as a reticulum cell sarcoma or an endothelioma. The patient failed rapidly, dying shortly after admission.

At autopsy multiple neoplastic masses, soft, friable and cellular, were found in many situations, notably the neck, the left lung and pleural cavity, the diaphragm and mediastinal lymph nodes, the heart and pericardial cavity, the cardiac portion of the stomach, the kidneys, the liver, the pancreas and both testes. The last-named organs weighed 100 Gm. each. Histologically the tumors were composed of small round cells, which were believed to be lymphocytes. The neoplasm was therefore a lymphosarcoma.

Because of the size of the testes and their early involvement, these organs were believed to be the primary seat of the tumor. With this opinion, however, other pathologists were not in accord, since there was no "normal lymphoid tissue in the testes from which such a tumor might originate." The probable site of the primary tumor was the lymphoid tissue of the neck.

MALIGNANT RENAL NEOPLASM FROM REMNANT OF THE MESONEPHRON. J. J. CLEMMER.

A white man aged 51 entered the hospital complaining of the passage of clots of blood during micturition. Hematuria had occurred at intervals for four months. A large immobile mass was found in the left upper quandrant of the abdomen. Urinalysis demonstrated hematuria. Ureteropyelograms showed complete obliteration of the left renal pelvis, believed by the roentgenologist to be due to a tumor of the left kidney. After one week the patient left the hospital against advice.

He was readmitted a year and a half later. During the interval he had had occasional hematuria. For a month or more he had suffered from a cough with mucoid sputum but no blood. Dyspnea and a feeling of fulness with frequent regurgitation were other complaints. There was physical and roentgenologic evidence of tumor metastases to the lungs, peritoneum, cervical lymph nodes and scalp. The patient died two weeks after the second admission.

At necropsy a large retroperitoneal tumor measuring 30 by 14 by 19 cm. was found in the site of the left kidney and adrenal gland. These organs could not be identified grossly, but the left renal artery and vein and the left ureter were traced into the mass. The tumor was solid, but contained foci of necrosis, hemorrhage and calcification. Metastatic nodules were present in the lungs, right kidney, myocardium, pancreas, peritoneum, scalp and cervical and retroperitoneal lymph nodes.

Microscopic study of the mass showed it to be entirely neoplastic with the exception of a narrow peripheral zone of dense scar tissue in which were embedded scattered glomeruli, many of them hyalinized. The tumor tissue, primary and metastatic, exhibited marked histologic variation; areas resembling renal tubules, adrenal cortex, ovarian papillary cystadenocarcinoma and fibrosarcoma were demonstrable.

The neoplasm is believed to have arisen from a remnant of the mesonephron, probably located in the left kidney. Hansmann and Budd described a series of "massive unattached retroperitoneal tumors" which, they concluded, arise from remnants of the urogenital apparatus. Such embryonic remnants may occur also within the adult urogenital organs. Histologically the reported neoplasm is similar to that group which Hansmann and Budd thought to be of mesonephric origin.

A Solitary Nonparasitic Cyst of the Liver. A. W. Wright.

A 28 year old white woman was admitted to the hospital complaining of dull pain and a mass in the abdomen. The mass was located in the right upper quadrant, was quite large and moderately firm. A diagnosis of possible tumor of the gallbladder was made, and operation was performed.

A large fluctuant thin-walled cyst attached to the undersurface of the right lobe of the liver was removed. Its outer surface was pinkish white and smooth except at the point of its attachment to the liver, which was a roughened area measuring approximately 8 by 2 cm. The cyst was considered to be a greatly dilated gallbladder. It was removed with little difficulty.

The structure measured 19 by 16 by 8 cm. Its wall was thin, translucent, membranous and tough. On the undersurface of the cyst and appearing to be an integral part of its wall was the elongated, narrow gallbladder. The interior was an irregular smooth-surfaced saccular space, which was divided into smaller compartments by weblike bands of connective tissue. There was no communication between the gallbladder and the cystic space. Histologically the wall was composed of dense fibrous tissue, which was congested and chronically inflamed. The lining cells were flat and differed markedly from those lining the cavity of the gallbladder. There was no evidence of hepatic tissue in any part of the structure.

Solitary nonparasitic cyst of the liver is rare. It is found usually in middle or late middle life, and women are most commonly affected. It may be congenital or it may occur as a result of inflammation or direct injury. In most cases it is congenital, arising from cystic dilatation of an aberrant bile duct. Of this group the present case is believed to be an example.

PATHOLOGICAL SOCIETY OF PHILADELPHIA

Feb. 13, 1936

Esmond R. Long, *President*

Herbert R. Ratcliffe, *Secretary*

Actinomycosis of the Liver with Involvement of the Abdominal Wall. Abraham Trasoff and David Meranze.

A tailor 67 years old was admitted on April 9, 1934, to the surgical service of the Mount Sinai Hospital because of a swelling in the epigastrium of twenty years' duration which had become painful five days prior to admission. Below the xiphoid process there was a tender reddened induration of the skin with fluctuation in the center. A deep incision brought out a considerable amount of pus. Roentgenograms of the abdomen and stomach showed nothing of importance. He was referred to the outpatient department, where a diagnosis of actinomycosis was made and confirmed. Iodides were administered, and irradiation was applied to the indurated area. In spite of this the wound failed to heal. He was readmitted on March 28, 1935, nine months later, with asthenia, loss of about 100 pounds (45.4 Kg.), anorexia, belching and pain in the right costal margin. He presented marked cachexia and pallor, a superficial lymph adenopathy, a phthisical condition of the chest with evidence of fibrosis, poor functioning of the myocardium of the arteriosclerotic type, a scaphoid abdomen with a draining wound below the xiphoid process and a tender hard irregular mass in the right upper abdominal quadrant and epigastrium. The temperature was 102 F., with the pulse and respiratory rates in proportion. The blood pressure was 118 systolic and 70 diastolic. There was marked hypochromic anemia with a polymorphonuclear leukocyte count of 19,000. A smear of pus from the discharging sinus showed no actinomycetes. A study of liver function gave the icteric index as 7.8 and urobilinogen as 1:20;

a test of galactose tolerance yielded 1 Gm. in six hours. Gastric analysis revealed no free hydrochloric acid, but ferments were present. On three examinations with a barium sulfate meal a constant irregularity in the pylorus was noted. In spite of supportive measures the patient died one month after admission. Post mortem there was found a sinus in the region of the epigastrium, from which pus could be expressed. The skin and fascia in the upper part of the abdomen showed a marked inflammatory reaction and were infiltrated with purulent material. This extended throughout the entire abdominal wall at this level and between the leaves of the diaphragm, which was thickened and firmer than normal. The liver and the subdiaphragmatic area at the right lobe were completely walled off from the rest of the abdominal cavity by adhesions, which matted together and distorted the intestines and omentum at this point. The liver was enlarged and contained one large abscess cavity and many smaller abscesses, occupying chiefly the medial half of the right lobe. The cavities were incompletely filled with necrotic grayish material. The surrounding liver tissue was involved in an inflammatory reaction. The involvement did not penetrate the anterior and posterior surfaces. The stomach was distended in the pyloric region and was adherent to the base of the liver. As a result the pyloric region was pulled upward and distorted. This probably accounted for the deformity noted on roentgen examination. The other organs showed arteriosclerosis, fibrosis and congestion.

SYMPATHICOBLASTOMA OF THE NECK SIMULATING FOR A TIME HODGKIN'S DISEASE. PHILIP D. GILBERT.

The onset occurred in June 1934 with the appearance of a walnut-sized tumor below the left mastoid process. In six weeks it increased to the dimensions of a hen's egg. Following tonsillectomy in September, the mass entirely disappeared only to reappear one month later and progress rapidly. The patient, a Negro aged 15, was admitted on two occasions to a local hospital, first in February and again in May of 1935. On each admission the only notable features were a large mass on the left side of the neck, palpable epitrochlear nodes, evidence of marked loss of weight, moderate secondary anemia and an erythrocyte count varying between 3,000,000 and 3,800,000, with the leukocytes numbering 18,000 per cubic millimeter. A biopsy of a node was made, and the final consensus was that the lesion was a granuloma suggestive of Hodgkin's disease. On each occasion the youth was treated with roentgen irradiation of the left side of the neck, without response, injections of a mixture of erysipelas and prodigiosus toxins (Coley) up to 17 minims (1.04 cc.) and blood transfusions. A second biopsy in another hospital was reported as showing transitional cell sarcoma. On July 31 the patient was admitted to the Philadelphia General Hospital suffering from intractable pain in the neck. Roentgenograms of the chest, skull and spine showed no evidence of metastatic tumor. There were paresis of the rectus muscle of the left eye, loss of tactile and pain sense in the distribution of the left fifth nerve, and difficulty and pain in swallowing, associated with dysphonia. The condition became progressively worse, and after an illness lasting approximately eighteen months the patient died, on Dec. 7, 1935.

The previous biopsy was reviewed and reported as follows: "The ill defined tumor appears to be composed mainly of large cells containing extremely vesicular nuclei, small nucleoli and poorly defined cytoplasm, which sometimes forms almost a syncytium; an occasional cell with definite cytoplasmic tendrils resembles a ganglion cell. Admixed with the larger cells are smaller elements, hardly separable from lymphocytes, sometimes accumulated in well demarcated sheets in which transition through small to large spindle-shaped forms can be distinguished. Very striking is the presence of extremely thick-walled vessels, the walls containing small round to spindle-shaped cells of this same variety."

The diagnosis was: sympathicoblastoma (peripheral neurogenic tumor) of the neck.

A necropsy was done three hours after death. A hemispheric tumor, apparently encapsulated, was found in the left side of the neck, forming a collar about the

common and external carotid arteries, the lumen of the latter being narrowed by the compression. By direct extension the tumor had invaded the base of the skull on the left side and completely filled the entire left middle fossa, replacing the left gasserian ganglion and a portion of the pituitary fossa and compressing the middle cerebral artery, which resulted in anemic necrosis of the portion of the cortex supplied by the superficial branch. Cranial nerves V to XII were adherent to the tumor on the left. Portions of the spine and the lamina of the first cervical vertebra, the heads of the first, second, third and fourth ribs on the right and the body of the seventh rib on the left were replaced by the tumor.

HEMOCHROMATOSIS WITH PRIMARY CARCINOMA OF THE LIVER. O. NORRIS SMITH.

The 60 year old white man presented was first studied in 1932 at the Hospital of the University of Pennsylvania. At that time he was suffering from a massive hematopericardium, which developed after a trivial thoracic injury. The liver was considerably enlarged and tender. The blood pressure was normal. A year later the liver was found enlarged and firm but not tender. The systolic blood pressure averaged 210 mm. In March 1935 hypertension, hepatic enlargement and diffuse brownish pigmentation of the skin were present, and, in addition, there was a diabetic sugar tolerance curve. Marked ascites developed, and the man died in the fall of the same year.

The diagnosis of hemochromatosis was confirmed at autopsy. The liver, spleen, pancreas and many lymph nodes were diffusely reddish brown. In addition to the pigment changes, the liver was firm, irregularly nodular and considerably enlarged, weighing 3,310 Gm. On the cut surfaces the blood vessels were found to be dilated with a slimy green material. The pancreas was small and firm.

Microscopic examination revealed massive deposition of hemosiderin and lesser amounts of hemofuscin in many viscera. The green mass proved to be a primary adenocarcinoma of the intrahepatic bile ducts, which had invaded the hepatic vessels. Atrophy, fibrosis and pigmentation of the pancreas were associated with hyalinization of the islands of Langerhans.

Analysis of the 160 odd reported cases of hemochromatosis shows an association with primary carcinoma of the liver in approximately 10 per cent of the cases. However, it must not be thought that hemochromatosis as such is responsible for this relatively high incidence, for, in a series of cases of Laënnec's and other types of cirrhosis studied at the Cook County Hospital in Chicago, associated primary carcinoma of the liver was also found in approximately 10 per cent of the cases.

CARCINOMA OF THE KIDNEY IN FROGS. BALDUIN LUCKÉ.

Adenocarcinoma of the kidney is common in leopard frogs (Lucké, Balduin: *Am. J. Cancer* **20**:379, 1934; **22**:326, 1934). In a large proportion of the tumors many of the cells have acidophilic intranuclear inclusions, a fact which may be regarded as presumptive evidence of the activity of a virus. Otherwise these tumors closely resemble carcinoma in man both in structure and in invasive tendency; metastasis, though less frequent than in most forms of human carcinoma, has been observed in a considerable number of cases.

The present report deals with the results of the first series of experiments in transmission of the tumor and with the behavior of the tumor in cultures.

Transplantation Experiments.—Over 1,400 frogs have been examined, approximately half of which received material from a number of different tumors by various routes (intramuscularly, intracranially, into the lymph sacs, into the pleuroperitoneal cavity) and in various forms (solid grafts, suspensions of tumor cells and cell-free desiccates); the other half were kept under identical conditions to serve as controls. Growths at the sites of inoculation were rare and soon retrogressed. However, tumors developed in the kidneys of many of the frogs. These renal tumors were morphologically identical with the spontaneous carcinoma; their occurrence did not depend on the particular site of inoculation; their frequency

increased with the period of survival of the animals. Thus, in frogs which died within three months after inoculation the incidence of renal tumors was approximately the same as in the control group, i. e., less than 2 per cent. The incidence reached 25 per cent in the group that survived over one year.

These results are consistent with the hypothesis that with inoculation of tumor material an agent has been transmitted which is organ-specific and probably of the nature of a virus.

Tissue Culture.—Tissue cultures made in a medium of frog plasma, chicken plasma and chick embryo extract grew rapidly, the outgrowths forming broad membranous sheets. The cells and their nuceli varied considerably in size, and mitotic figures were fairly common. This is believed to be the first tumor from a cold-blooded animal that has been grown in tissue culture.

March 12, 1936

ESMOND R. LONG, *Presiding*

RECURRENT RENAL TUBERCULOSIS AFTER NEPHRECTOMY. MAURICE S. JACOBS and B. A. GOULEY.

A woman aged 47 had right nephrectomy in 1912, two operations on the bladder in 1914, right oophorectomy and appendectomy in 1920, and ventral herniorrhaphy in 1923. The familial history was negative for tuberculosis. The blood pressure was 250 systolic and 150 diastolic. The pulse rate was 90. The eyegrounds indicated moderate sclerosis. The heart showed slight enlargement to the left, but there were no murmurs. The aorta was slightly widened. An electrocardiogram demonstrated marked left axis deviation. The Wassermann reaction of the blood was negative. There was moderate anemia. The specific gravity of the urine was 1.010; there was a cloud of albumin, and from 15 to 25 leukocytes with a few clumps were observed microscopically. The urea nitrogen of the blood varied between 98 and 156 mg. per hundred cubic centimeters. Cystoscopy revealed a violent infection of the bladder and pelvis. Following cystoscopy under gas anesthesia, the oliguria was improved so that the patient was in water balance for several days. Coincidentally with the relief of the cystopyelitis, the urea nitrogen of the blood dropped to 44 mg. per hundred cubic centimeters, and the patient was clinically improved. However, the symptoms again became aggravated, nausea and vomiting set in, the urea nitrogen of the blood rose to 70 mg. per hundred cubic centimeters, and on Jan. 15, 1936, the patient died.

Autopsy revealed the left kidney about one and one half times the normal size. The upper pole was replaced by a huge tuberculous abscess, which exuded foul-smelling pus on incision. The lower pole was one large single cyst, with complete destruction of the tissue of the kidney. The midsection was the only part resembling normal tissue, though this was obviously diseased, and, on squeezing this area, pus exuded, which showed tubercle bacilli on direct smear. The left ureter was very much thickened and dilated.

The heart showed slight hypertrophy of the left ventricle, though in general it was contracted. There were focal hemorrhages throughout the gastro-intestinal tract, as is usually seen in uremia. There were no signs of old or recent tuberculosis in the lungs or abdomen. There was no embolus or thrombus in the heart or brain. The cause of death seemed to be uremia, the heart having stopped in systole.

THE MECHANISM OF IMMUNITY IN TUBERCULOSIS: THE RÔLE OF SOME CELLULAR AND EXTRACELLULAR FACTORS. MAX B. LURIE.

If a mixture of tubercle bacilli and melted agar is injected subcutaneously into normal rabbits, the bacilli grow unhindered in the acellular agar islands at a great distance from the cells. This is brought about by the body fluids which

penetrate the agar. In BCG-vaccinated or tuberculous rabbits the bacilli are markedly inhibited in their growth in these islands. The extent of growth was determined by culture. The agar focus as a whole and the individual agar islands of the sensitized animal become surrounded by an extensive and dense network of fibrin, while in the normal animal this barrier is less pronounced. Furthermore, both tubercle bacilli and particulate matter tend to be agglutinated in the acellular agar of the immunized animal, whereas in the normal animal both the bacilli and the carbon particles are largely dispersed. Again, in the normal animal the polymorphonuclears, which have no capacity to destroy tubercle bacilli, persist in the focus for a long time, and the mononuclears, the capacity of which for destroying tubercle bacilli is slight, are tardy in their appearance. In the vaccinated or tuberculous rabbit, on the other hand, the polymorphonuclears soon disappear from the site of reinfection, and their place is taken by the rapidly mobilized mononuclear phagocytes, the capacity of which to destroy tubercle bacilli is greatly enhanced.

These cellular and extracellular factors are effective in confining small doses of reinfection at the portal of entry. With large doses of reinfection the increased lymph flow resulting from the intensified inflammation in the immunized animal brings about a more rapid dissemination of the bacilli to the draining lymph nodes than in the normal animal. The most significant factor in immunity is the increased capacity of the mononuclear phagocytes to destroy tubercle bacilli. The invading bacilli that reach the draining lymph nodes of the immunized animal are retarded in multiplication or destroyed by these phagocytes.

Thus immunity in tuberculosis rests on factors which inhibit the growth and immobilize and destroy the bacilli of reinfection. What are the factors responsible for these differences?

All attempts thus far to demonstrate in vitro the extracellular factor responsible for the inhibition of growth of the bacilli in the vaccinated animal have met with no success.

The agar focus and the pleural exudate of tuberculous rabbits in response to injection of dead tubercle bacilli are consistently more acid than the corresponding agar focus and pleural exudate of normal rabbits. The p_H difference, however, is on the average only 0.2. This slightly more acid reaction of the agar focus of the vaccinated animal is obviously insufficient to explain the marked inhibition of growth observed in the vaccinated animal. Possibly the enzymes released by the mononuclears of the tuberculous rabbit into the agar may explain the results. The more rapid mobilization of the mononuclears in the tuberculous rabbit is apparently a nonspecific change in their reactivity or in the permeability of the blood vessels, for the same observation is made in the pleural exudate of tuberculous rabbits in response to the injection of aleuronat and starch. There is no consistent correlation between the p_H of an exudate and the leukocytic formula.

The agglutination of tubercle bacilli and carbon particles in the tuberculous rabbit is apparently a property of the plasma.

CERTAIN PATHOLOGIC CHANGES IN THE HEART IN SCARLET FEVER. LAWRENCE W. SMITH.

An attempt has been made to present the essential microscopic pathologic changes which were observed in a series of eighty-eight cases of scarlet fever and related hemolytic streptococcic infections. These changes seem to be of a toxic nature and are progressive. It is not possible to distinguish any essential difference between the early proliferative and inflammatory changes of the myocardium in the development of the Aschoff body in rheumatic fever from these same microscopic alterations in the myocardium in scarlet fever. As a result, the belief is advanced that the hemolytic streptococcus is a nonspecific infectious agent, infection with which may result, on the basis of the constitutional diathesis of the patient, in either a simple inflammation of the throat with or without the development of a typical scarlatiniform rash or in the development of a typical rheumatic condition.

PATHOLOGIC FEATURES FOLLOWING SHOCK WITH DELAYED DEATH. VIRGIL H. MOON and DAVID R. MORGAN.

Confused discussion has resulted from vague conceptions as to what constitutes shock. The condition has not been defined satisfactorily, and unrelated conditions are frequently included in the discussions. We have reported on the occurrence and pathogenesis of the shock syndrome and have demonstrated its characteristic morbid anatomic changes. A survey of recent literature indicates that most of the articles on shock pertained to this syndrome. The physiologic features reported in such articles may be formulated into a useful conception of the condition:

Shock is a circulatory deficiency, not cardiac and not vasomotor in origin, characterized by decreased volume of blood and cardiac output and by hemoconcentration. The latter feature appears early, is easily determined and is seen in cases of shock of moderate severity before variations in blood pressure occur. For these reasons it is most useful as an indicator. The following features are regularly associated when the syndrome is fully developed: lowered blood pressure and basal metabolism, diminished renal excretion and increased cardiac rate. In advanced stages the coagulability of the blood is diminished, the blood chlorides are below normal and the nonprotein nitrogen is increased.

The shock syndrome occurs in varying degrees and with varying rapidity. When shock leads to immediate death, diffuse congestive features are seen in the viscera. The serous surfaces and the mucosae have a purplish color and contain ecchymoses. Blood-tinged fluid is present in the serous cavities. The liver and kidneys are deeply congested, but the spleen is contracted, dry and bloodless. The lungs are diffusely congested and contain many capillary hemorrhages. When death is delayed twenty-four hours or longer, marked edema develops in the lungs and mucosae. The edema fluid has a high protein content and a specific gravity closely approximating that of the blood serum.

Shock with delayed death, accompanied by pulmonary edema, was produced by various simple procedures: by the intraperitoneal introduction of muscle substance, burns, intestinal obstruction, the injection of bile or bile salts, the administration of barbiturates to secure deep narcosis and by injections of histamine. In each case marked hemoconcentration developed, and the tissue changes characteristic of shock were present. Marked pulmonary edema was a feature in each instance.

Lungs in which the circulation is impaired and the spaces are filled with edema fluid are predisposed to pneumonia. When death was delayed several days, secondary pneumonia developed regularly. It had the same characteristics as the terminal pneumonia seen in man following extensive trauma, burns, surgical operations, intoxications and severe infections.

Pulmonary edema of the type described results from increased vascular permeability and is accompanied by hemoconcentration and circulatory inefficiency. The mechanism of such edema is integral with that of the shock syndrome. A common type of terminal pneumonia develops if neither death nor recovery occurs soon.

Book Reviews

Manuel de biochimie. By Pierre Thomas, de l'Institut Pasteur, Professeur de Chimie biologique à la Faculté de médecine de Cluj. Cloth. Price, 180 francs. Pp. 978, with 52 illustrations. Paris, Masson & Cie, 1936.

This book is addressed to physicians, biologists and others to whom a knowledge of biologic chemistry may be considered indispensable. It is divided into four sections. The first section deals with the physical chemistry of cells and organisms and comprises one fourth of the volume. This section, which serves to distinguish this volume from practically all other textbooks of biochemistry, is the most interesting and unusual in the whole book. In it Thomas has chosen for discussion those physicochemical concepts which have been found especially useful in the interpretation of biochemical processes. There are fifteen chapters dealing with such important matters as atoms and molecules, colloids, adsorption, surface tension, viscosity, osmotic pressure, catalysis, electrolytic dissociation, biologic significance of the colloidal state, permeability and enzymes. The second section of the book, on the chemical constituents of the cell, contains ten chapters on proteins, carbohydrates, lipids and mineral salts. The third section, on synthesis, hydrolysis and desmolysis, contains eight chapters which cover the syntheses of various substances in nature and the transformations which these substances undergo in the body during metabolism. The last third of the book consists of fifteen chapters on the tissues and their function. These chapters deal with such subjects as the blood, vitamins, hormones, muscle and muscular contraction, the nervous system and sense organs, the sex organs, urine and plant substances. At the end of each chapter the author has added some practical exercises. In general, however, these exercises do not cover the field of biochemistry as thoroughly as is done in the better laboratory manuals of this country. The book is beautifully printed and bound, is up to date and contains a great deal of material not ordinarily found in biochemical texts in English. It is a useful and stimulating volume and should find a distinct place in the libraries of biochemists and others interested in obtaining a broad understanding of the chemical reactions underlying life processes.

The Harvey Lectures. Delivered under the Auspices of the Harvey Society of New York, 1934-1935, under the Patronage of the New York Academy of Medicine. By Dr. W. Bosworth Castle, Dr. William Cumming Rose, Dr. Wilbur A. Sawyer, Dr. Alfred N. Richards, Dr. E. C. Dodds, Dr. G. V. Anrep, Dr. Francis G. Blake, Dr. John H. Northrop. Series XXX. Cloth. Price, $4. Pp. 270, with illustrations. Baltimore: Williams & Wilkins Company, 1936.

This volume contains the thirtieth series of Harvey lectures. The lecturers and their topics are as follows: William Bosworth Castle, of Harvard University Medical School, discusses the etiology of pernicious and related macrocytic anemias; William C. Rose, of the University of Illinois, treats of the significance of the amino-acids in nutrition, and Wilbur A. Sawyer, of the Rockefeller Foundation, discusses the present distribution of yellow fever and its significance. Alfred N. Richards, of the University of Pennsylvania, considers the processes of urine formation in the amphibian kidney, and E. C. Dodds, of the Middlesex Hospital, London, the specificity of hormonal and other biologic reactions. G. V. Anrep, of the Egyptian University, discusses the relation of the circulation in voluntary and plain muscle to activity; Francis G. Blake, of Yale University, pneumothorax in the treatment of pneumonia, and John H. Northrop, of the Rockefeller Institute

for Medical Research, Princeton, the isolation and properties of crystalline pepsin and trypsin. These lectures are lucid presentations of important recent advances in medical science.

Pathology and Microbiology, Being Mainly Proceedings of the Chinese Society of Pathology and Microbiology Held in Canton, November 5-8, 1935. Chinese Medical Journal, Supplement 1. Paper. Price, $2.50. Pp. 518, with 75 plates. Peiping: Chinese Medical Journal, 1936.

Owing to the increasing number of papers on subjects in the fields of pathology, bacteriology and parasitology submitted to it, the *Chinese Medical Journal* has started a series of supplements for the publication of such material. This supplement, the first of the series, is of the same size and format as the regular issues of the journal. The field of pathology is represented by thirteen articles, that of bacteriology by twenty-three and that of parasitology by fourteen. At the end is a valuable paper by R. J. C. Hoeppli on the methods of illustrating scientific papers. The articles, all of which are of interest and value, embody the results of investigative work in the following institutions: Peiping Union Medical College, Moukden Medical College, Hsiang-Ya Medical College, the Henry Lester Institute of Medical Research (Shanghai), the National Epidemic Prevention Bureau (Peiping) and the Central Field Health Station (Nanking). The editorial style, the presswork, the illustrations and the paper compare favorably with those of similar publications in this country. The supplement illustrates well the high quality of the scientific work in Chinese institutions in the fields represented.